STUDIES OF 2- AND 8-SUBSTITUTED PURINES INCLUDING RELATED INTERMEDIATES

A Thesis

submitted to the

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Master of Science

by

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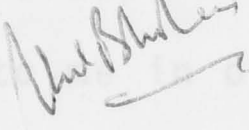
CONTENTS

The work described in this thesis was carried out by the candidate at the Australian National University.
Where the work of others was employed appropriate references have been given.



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Thanks are due to the Australian National University for the Scholarship award.

Last, but not least, I take this opportunity to thank Mrs S.M. Schenk who typed this thesis with efficiency and considerable patience.

Nomenclature

Nomenclature used in this thesis conforms essentially to that of I.U.P.A.C. (as adopted by the Chemical Society). The only divergence made has been in the case of theophylline derivatives. With these compounds substitutive forms of the trivial name have been used for clarity and convenience in preference to the more unwieldy systematic nomenclature.

Summary

The syntheses and reactions of some 2- and 8-substituted purines are reported in this thesis.

Part I is concerned with work relating to the direct C-8 alkylation of purines, in particular, reactions involving theophyllines. Interest in this topic arose from observations made of the biological action of aromatic carcinogens in which the latter have been shown to form covalently linked adducts with the 8-position of the purine bases in DNA. Studies were initiated with the aim of gaining some insight into the conditions which would favour the formation of 8-alkylpurines by direct attack at the 8-position of the purine.

Initially, two alkylation experiments in the literature were re-examined. In these, the action of benzyl and crotyl halides on theophylline was reported to have given the corresponding 8-alkyl-theophyllines among the products. The benzylation experiment, when repeated under the reported conditions, gave in addition to the expected 7-benzyltheophylline small amounts of 8-benzyl and 7,8-dibenzyltheophylline. This result substantiated the fact that the direct C-8 alkylation of theophylline under the alkaline alkylating conditions does occur to some extent.

In the light of current publications which describe alkylation at the 8-position of purine by alkyl radicals, comparative studies have been carried out on the benzylation of theophylline under continuous irradiation by u.v. light. Under these conditions, which do not preclude the possibility of benzyl radicals being present, preliminary results show that the yield of 8-benzyltheophylline is significantly increased. From these data it is postulated that the formation of the 7,8-dibenzyltheophylline occurs through a radical benzylation at the 8-position which is then followed by a thermally controlled alkylation at the 7-position.

The reaction of crotyl bromide with theophylline has been similarly investigated. When the original alkylation was repeated the findings of the original workers, who reported that only 8-crotyltheophylline was formed, were confirmed.

A further example of C-8 alkylation arises from the methylation of 2-methylthiopurine with diazomethane which has been claimed to give as a minor product the dimethylated derivative, 7,8-dimethyl-2-methylthiopurine. While the minor product is undoubtedly formed through an 8-methylation of 2-methylthiopurine the above derivative has been ruled out as a candidate by an unambiguous synthesis made of this particular purine.

On the synthetic aspects considerable endeavours have been made towards the synthesis of various benzyl derivatives of theophylline encountered in the benzylation studies. From this work has emerged quite a number of interesting observations on the unexpected behaviour of benzyl groups attached to either pyrimidine intermediates or to purines. These include removal, rearrangement and involvement of such groups in ring-closure reactions.

Part II deals with the syntheses of some 2-cycloalkylamino-6,9-dimethylpurines which were required for biological testing as amplifiers of phleomycin. 6,9-Dimethyl-2-(piperidin-1-yl)purine was found to be the most active of the purines which had been synthesised for the programme. From the data obtained with the purines tested so far, no rationale can be given for the effects of structure variation on the biological activity of the purine derivatives.

Part III of this thesis is devoted to exploring the versatility of tetraethoxymethane, and to a lesser extent the tetramethoxy and tetrapropoxy analogues, as cyclising agents in the Traube synthesis of 8-alkoxypurines from 4,5-diaminopyrimidines.

Also, the mode of cyclisation of 4,5-diamino-6-substituted-aminopyrimidines with tetraethoxymethane is compared with the products derived from use of orthoester type reagents. With the results obtained to date it has been found that the tetraalkoxymethanes behave more like the general cyclising agents and afford the appropriate 9-alkyl-8-ethoxyadenines rather than the isomeric 6-alkylamino-8-ethoxypurines as is the case with the orthoesters.

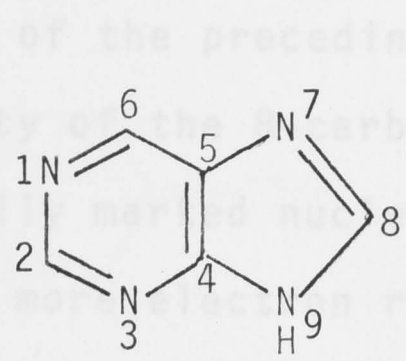
PART I. Studies Relating to Alkylation of Purines at the 8-Position

A. INTRODUCTION

General

Purines as a class of heterocyclic compounds are of considerable significance because of their occurrence in all forms of living matter. It is not surprising therefore that these compounds have been the subject of intensive biological, biochemical and chemical investigation.

The name and numbering of the purine ring (1) are due to Emil Fischer who first proved the structure



(1)

of the parent compound by an unambiguous synthesis (Fischer and Ach, 1897). Although both are still retained the numbering is, however, unsystematical as far as the general rules for fused heterocycles apply. Under these rules it is more precisely defined as an 7(9)H-imidazo[4,5-d]pyrimidine.

The ring system is essentially a combination of a π-electron deficient pyrimidine ring and a π-electron excessive imidazole ring. A resultant effect of this is an electron redistribution with the localisation of the π-electrons in the area of the

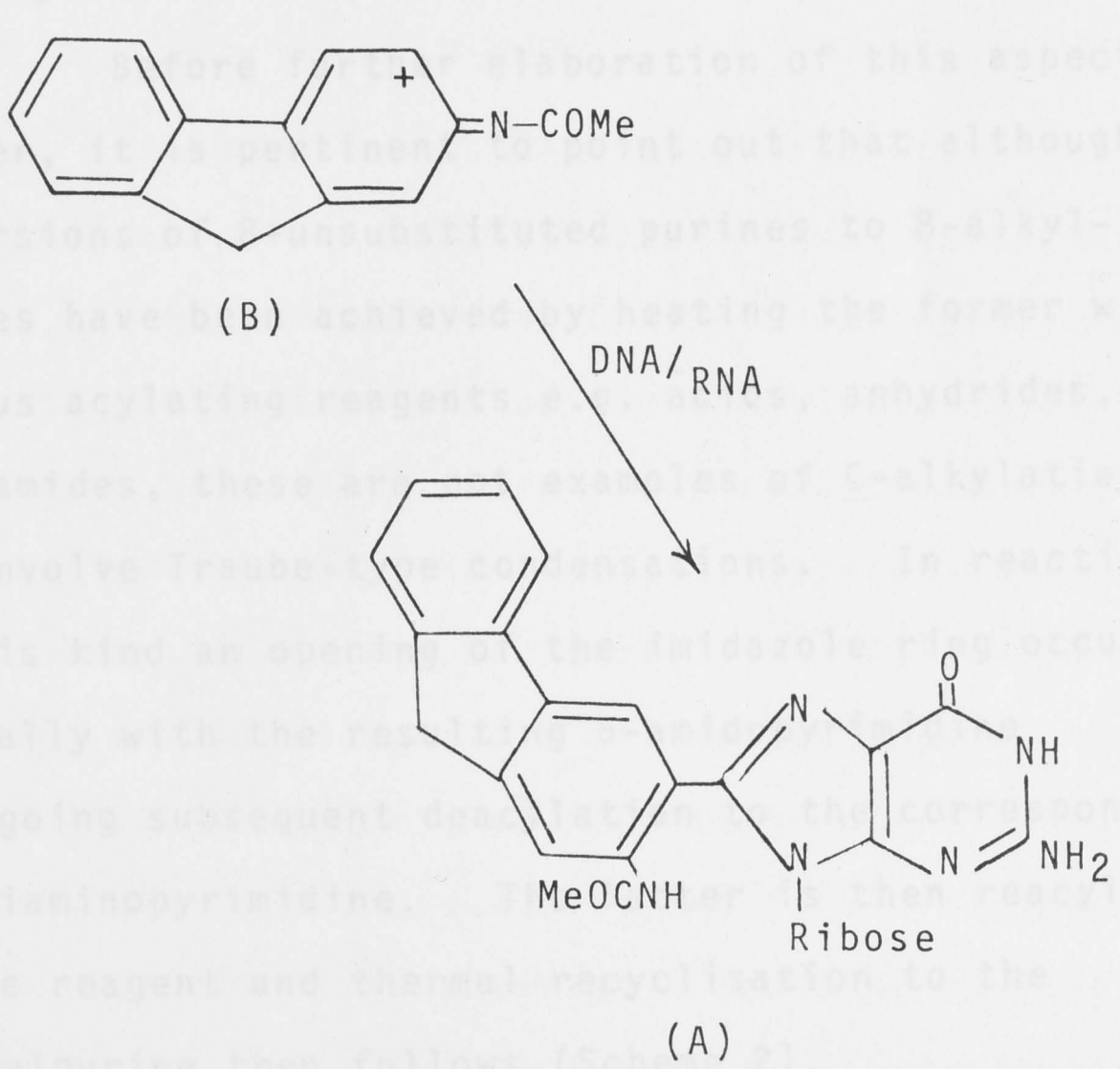
ring nitrogen atoms; the adjacent carbon atoms as a consequence show a great deal of electrophilic character. This property varies quite considerably with the position of the carbon atom in the ring and is also greatly influenced by the presence of other groups in the ring. Studies (Sutcliffe and Robins, 1963) carried out on nucleophilic displacement of chlorine atoms at the 2-, 6- and 8-positions indicate that for the unionised molecule, the 8-carbon atom seems to be the most electron deficient and substituents at this position are displaced first, the overall order of reaction being C-8, then C-6 and lastly C-2.

In spite of the preceding remarks concerning the electrophilicity of the 8-carbon atom this position can also show equally marked nucleophilic character. This occurs if two more electron releasing groups are present in the molecule, the effect of these being to compensate for the π-electron deficiency in the pyrimidine moiety and thereby restore the π-electron excessive character of the imidazole ring. Attack at C-8 by electrophiles now becomes possible, this being shown by the fact that direct halogenation, nitration and diazotisation can be effected with many purines.

Historical

The demonstrated nucleophilic character of the 8-position of some purines, noted above, has also biological significance when viewed in the light of reports which have appeared recently concerning the possible mode of action of aromatic carcinogens.

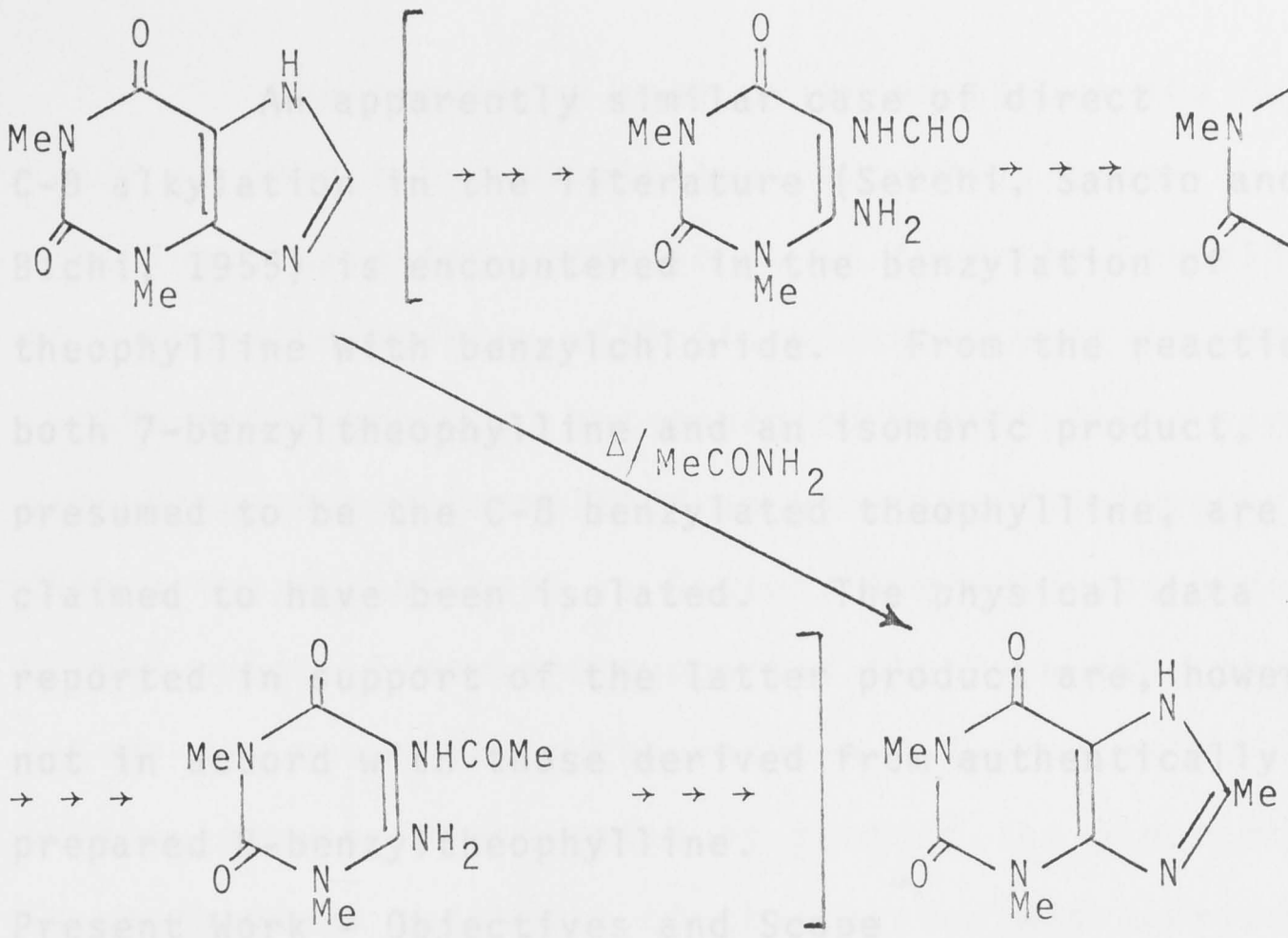
In these it is suggested that the carcinogen, in the form of carbocation, acts as an alkylating agent towards the purine bases, in particular guanine (Kriek, 1972), of the nucleic acid. The results of these studies, both *in vivo* and *in vitro*, show that alkylation at C-8 is the major reaction involving the guanine moiety. This is seen in the isolation of the stable carcinogen-purine adduct (A) when acetylamino-fluorene (B) is the carcinogenic agent used (Scheme 1).



Scheme 1

This case of C-alkylation, i.e. an apparent direct electrophilic attack at C-8, is not well supported by other such examples in the literature. On theoretical grounds this is unexpected as under the usual alkaline alkylating conditions an N-9(7)-unsubstituted purine undergoes reaction in the anionic form. In this event both nitrogen atoms and the 8-carbon atom of the imidazole ring would be expected, because of charge localisation, to show some nucleophilic character. In practice, however, examples of C-8 alkyl derivatives being formed as a result of N-alkylating procedures carried out in alkaline media are very rare.

Before further elaboration of this aspect, however, it is pertinent to point out that although conversions of 8-unsubstituted purines to 8-alkylpurines have been achieved by heating the former with various acylating reagents e.g. acids, anhydrides, or acid amides, these are not examples of C-alkylation but involve Traube-type condensations. In reactions of this kind an opening of the imidazole ring occurs initially with the resulting 5-amidopyrimidine undergoing subsequent deacylation to the corresponding 4,5-diaminopyrimidine. The latter is then reacylated by the reagent and thermal recyclisation to the 8-alkylpurine then follows (Scheme 2).



Scheme 2

Although it has been shown recently that alkyl radicals will smoothly convert 8-unsubstituted purines to the corresponding 8-alkyl homologues, there are in the literature one or two examples of C-alkylation which have taken place under conditions which favoured ionic rather than radical interactions. In one of these (Donat and Carstens, 1959) following treatment of the sodium salt of theophylline with crotyl bromide ($MeCH{=}CH\text{-}CH_2Br$), at room temperature in aqueous solution, the sole product obtained is 8-crotyltheophylline. In this reaction it is somewhat surprising to find the absence of any N-7 alkylation in view of the facility with which theophylline usually gives only a 7-alkyl derivative under alkaline conditions.

An apparently similar case of direct C-8 alkylation in the literature (Serchi, Sancio and Bichi, 1955) is encountered in the benzylation of theophylline with benzylchloride. From the reaction both 7-benzyltheophylline and an isomeric product, presumed to be the C-8 benzylated theophylline, are claimed to have been isolated. The physical data reported in support of the latter product are, however, not in accord with those derived from authentically prepared 8-benzyltheophylline.

Present Work - Objectives and Scope

As the aim of the present studies was to attempt to gain insight into the reaction conditions which would predispose towards C-alkylation of purines, the initial step was to check the authenticity of the results of the two alkylation attempts on theophylline, noted above.

Although the experimental conditions employed would be expected to favour an ionic type of mechanism operating, there are a number of current publications in which alkylation at C-8 by alkyl radicals is reported to be both facile and to give good yields. With this aspect in mind, comparative studies were also made in which continuous irradiation by ultraviolet light was carried out during the alkylation procedure. Under these conditions, which favour alkyl radical formation, an increase in 8-alkylpurine formation would be expected to occur.

The remaining case reported (Brown and Ford, 1969) of direct C-8 alkylation, occurring under alkaline alkylating conditions, is from the methylation of 2-methylthiopurine with diazomethane when in addition to the major product, the expected 9-methylated purine, a minor product, formulated as N,8-dimethyl-2-methyl-thiopurine is obtained. Because the evidence given by the original workers supported the idea that the latter was most likely the unknown 7,8-dimethylpurine this point was investigated as part of the programme. An unambiguous synthesis of this purine was carried out and a comparison made of the data from both dimethylated compounds.

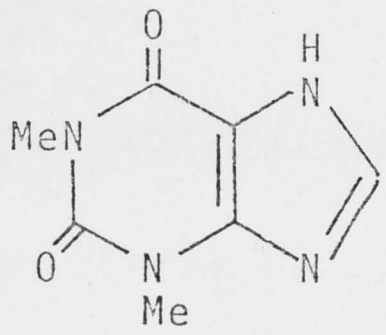
Considerable endeavours were made on the syntheses of the various benzyl derivatives of theophylline encountered in the work. In some cases, in spite of prolonged efforts, the required derivative was not obtained due to adverse and sometimes unexpected reactions occurring. Notable among these were a number of examples in which loss or rearrangement of benzyl groups was involved. An interesting example of this was encountered during the attempted synthesis of 8,8-dibenzyl-8H-theophylline. This derivative, if obtained, was expected to follow the reaction pattern of other 8,8-dialkyl-8H-purines and rearrange to the 7,8-dibenzyl analogue. However, in practice 8-benzyl-theophylline was the only product obtained; no 8,8-dibenzyltheophylline being detected.

At this stage, therefore, the scope of the work was broadened to include not only studies related to attack at C-8 of the purine but also investigations of the reactivity of the C-8 position towards the entry and departure of benzyl groups and the migration of benzyl groups located on imidazole ring nitrogen atoms. Inclusion of these additional topics in the programme was felt to be apposite to the problem of C-alkylation in view of cases of alkyl group migrations from C-8 to N-7 having been reported in purines. Thus, it was hoped that data from experiments in which group loss or rearrangement was experienced would, perhaps, throw more light on the mechanism of the C-alkylation reaction.

B. ALKYLATION STUDIES

a. Benzylation of theophylline

Serchi and coworkers (1955) carried out the benzylation of theophylline (1) with benzyl chloride in the presence of an equimolar amount of aqueous sodium bicarbonate using a temperature of around 100°. Alkylation of theophylline under these conditions normally gives only a 7-alkylated derivative but in this case, as well as the expected 7-benzyltheophylline (2) in 65% yield, a further product (22%) was obtained from the mother liquors. As the analysis of the latter showed it to be isomeric with the 7-benzyl derivative (2), it was suggested that it was 8-benzyltheophylline (3). This was a known compound which had been synthesised unambiguously by Hager and Krantz (1954) by fusing 4,5-diamino-1,3-dimethyluracil with phenylacetic acid and then cyclising the phenylacetamidopyrimidine in alkali (Scheme 3). A comparison of the physical data for the authentic purine with that reported by Serchi et al. for their isomer, however, showed no agreement. For example, the melting point of 8-benzyl-theophylline (289°) is some 40° higher than that reported by Serchi for his product. In addition, he reported that 7-benzyltheophylline and the isomeric derivative had the same R_f values in three different solvent systems; this was somewhat unusual considering the more polar nature, due to the ionisable proton in



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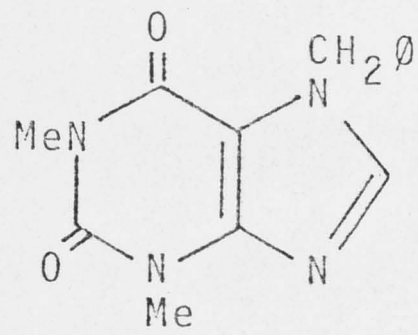

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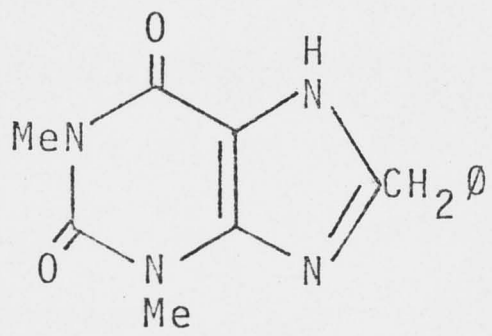

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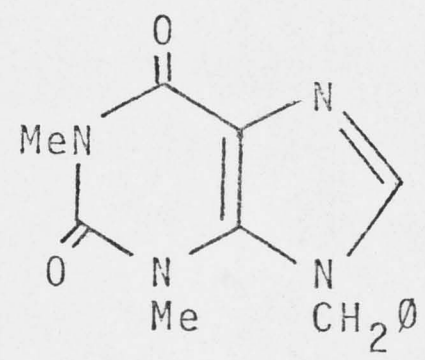

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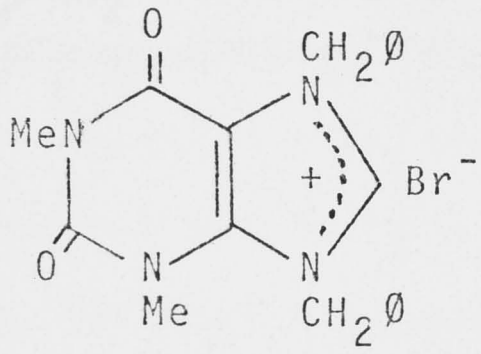

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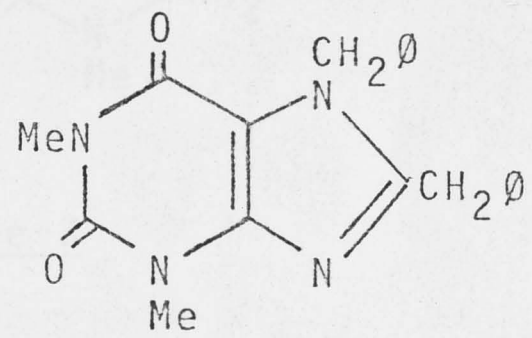

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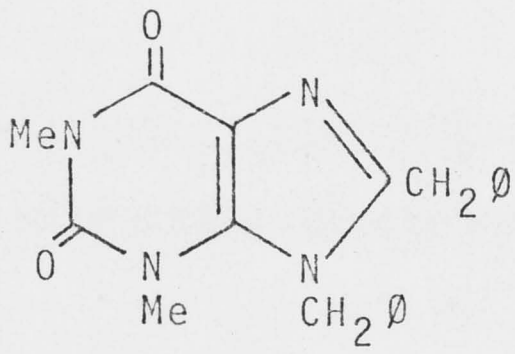

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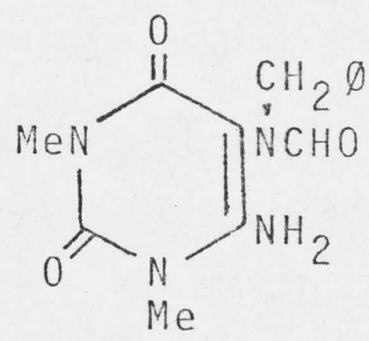

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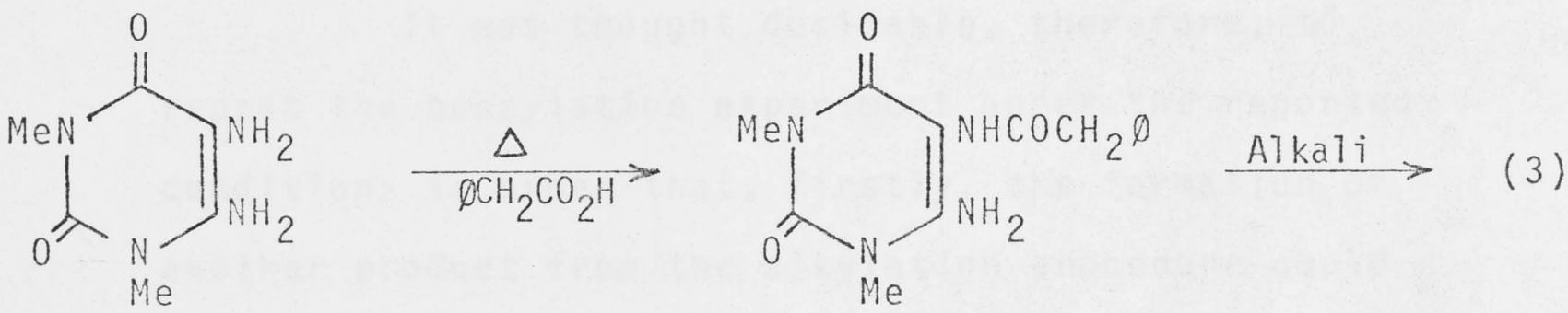


Scheme 3

the imidazole ring, of 8-benzyltheophylline. At no stage did Serchi and his coworkers appear to consider the possibility that the isomeric product was the 9-benzylated derivative (4). This attitude was, no doubt, influenced by the fact that no 9-alkyltheophylline has so far resulted from direct alkylation of theophylline. Conclusive evidence, however, that the unknown isomer was not the 9-benzylated purine (4) was provided the following year (Blicke and Schaaf, 1956) when this derivative was also unambiguously synthesised.

It was thought desirable, therefore, to repeat the benzylation experiment under the reported conditions in order that, firstly, the formation of another product from the alkylation procedure could be verified and, secondly, to ascertain, if such a product was formed, if this arose as a result of a C-rather than an N-alkylation reaction.

Following the alkylation of theophylline under the reported conditions the solid which separated out was then treated with alkali to dissolve any 8-benzyltheophylline present together with any unreacted theophylline; the residue, which was the major product, was recrystallised from ethanol giving the 7-benzyl derivative (2). From the alkaline filtrate a solid, identified as 8-benzyltheophylline (i.r., n.m.r., t.l.c.), was precipitated on acidification.

The mother liquors obtained from crystallisation of the alkali-washed crude reaction product, after suitable concentration, were run on a silica preparative t.l.c. plate, three bands being observed. Each band was extracted separately with hot chloroform and the extracts evaporated down. The product from the bottom band corresponded to unreacted theophylline, the middle one was due to 7-benzyltheophylline while the upper band gave an unknown product, m.p. 120-121°. Elementary analysis of this low m.p. material corresponded to that of a dibenzylated theophylline derivative. As this was not a salt, the quaternary form (5) could be excluded. Examination of the ^1H n.m.r. spectra revealed that the 8-H proton signal was absent from which it was inferred that one of the benzyl groups might be occupying this position. The location of the second benzyl group was, therefore, either at N-7 or N-9, with the former more favoured in view of the alkylation mode of theophylline in which the 7-alkylated analogue is always the preferred product. These suppositions were subsequently confirmed when the ^1H n.m.r. spectra (Figure 1, δ values in DMSO with TMS internal standard) of 7-benzyl-, 8-benzyl-, and 9-benzyl-theophylline were compared. In particular the chemical shifts of the benzylic protons ($-CH_2-$) due to the 7-benzyl (Figure 1a) and 8-benzyl derivatives (Figure 1b) showed the best correspondence with the two separate benzylic protons of the dibenzyl analogue (Figure 1d).

A further significant feature in identification of the position of the second benzyl group was the nature of the phenyl group proton signals. In both 7-benzyl- and 8-benzyl-theophylline the same singlet signal was shown; this also being the case with the dibenzylated purine, whereas in the 9-benzyl (Figure 1c) and 9-benzyl-8-substituted-theophyllines, extensive multiplicity was found. Chemical shifts for the above and related derivatives are given in Table 1. Thus, it seemed that the unknown derivative had the 7,8-dibenzyl (6) rather than the 8,9-dibenzyl (7) configuration. Virtually conclusive proof for the former assignment was the isolation of 7,8-dibenzyl-theophylline (6) when the sodium salt of 8-benzyl-theophylline (3) was treated with benzyl bromide. The two dibenzyl derivatives were shown to be identical in their m.p., i.r., ^{1}H n.m.r., u.v., t.l.c., and analytical data.

When the benzylation of theophylline was carried out at room temperature (12 h) mainly starting material was isolated; only trace amounts of 7- or 8-benzyl-theophylline could be detected by t.l.c.

In summation, therefore, it can be said that the repeat of the benzylation of theophylline, under the conditions reported by Serchi *et al.*, has not given the same results; no product with physical data corresponding to their "8-benzyltheophylline"

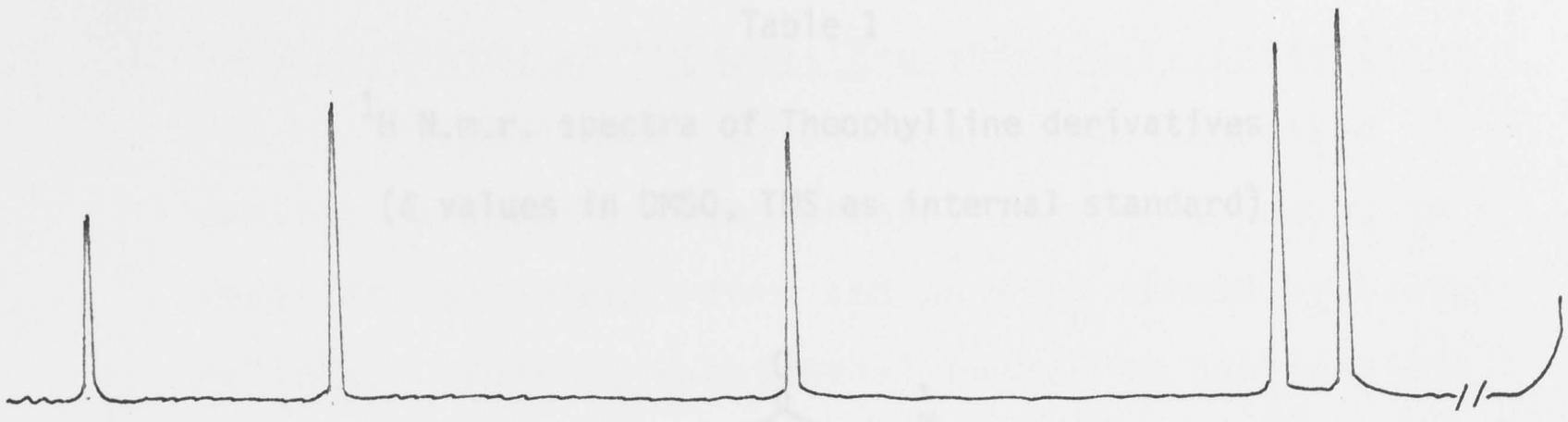
being isolated. In addition to the expected 7-benzyltheophylline (36%) the two minor products obtained were authentic 8-benzyltheophylline (0.8%) and a new derivative, 7,8-dibenzyltheophylline (0.4%).

Interpretation of the original workers' results in the light of the findings from the repeat benzylation experiments are difficult. The product erroneously claimed to be 8-benzyltheophylline would seem to be most likely an impure specimen, especially if we consider that the quoted m.p. from ethanol (248-249^{o}) rises to 256^{o} on recrystallisation from pyridine. These figures contrast sharply with the figure (289-290^{o}) given for authentic 8-benzyl-theophylline (Hager and Krantz, 1954). In view of the lack of further specific physical data, i.e. u.v., i.r., or ^{1}H n.m.r., for the unknown derivative it is suggested by way of explanation that some 8-benzyl-theophylline is present but this is contaminated with both the 7,8-dibenzylated homologue and unreacted theophylline, a fortuitous ratio of the last pair giving rise to the satisfactory analysis figures for the "monobenzylated" product.

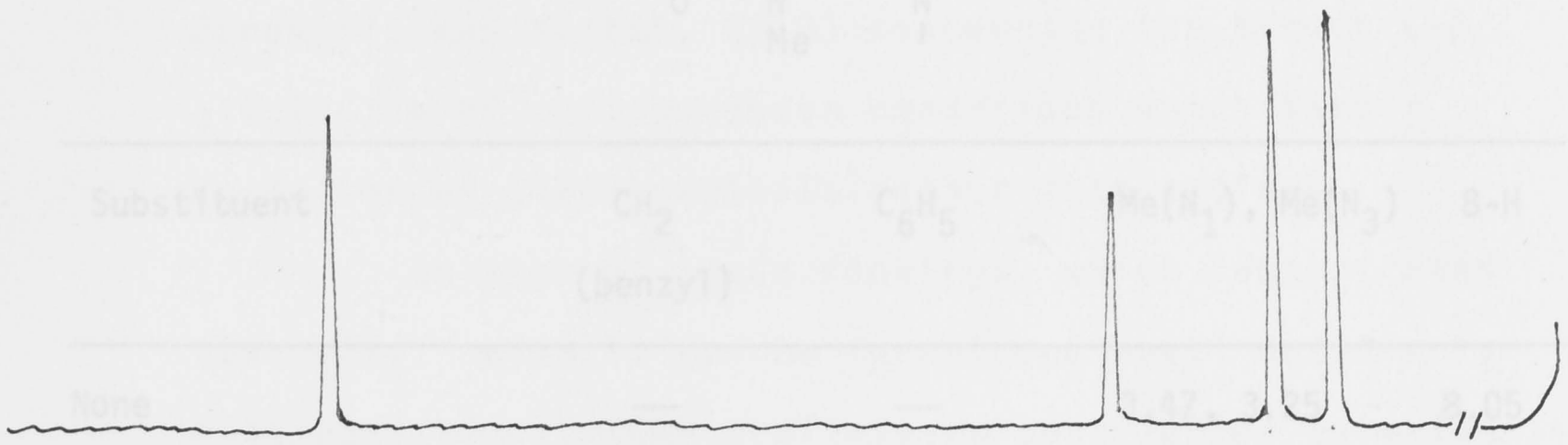
In view of the fact that the analytical data given by Serchi and coworkers were for monohydrated not anhydrous forms of the benzyl purines consideration was also given to the possibility that the unknown monobenzyl derivative was not a purine but 6-amino-5-(*N*-benzyl)formamido-1,3-dimethylpyrimidine-2,6-dione (8),

which has the same empirical formula. Under the alkaline reaction conditions this could easily arise through ring-opening of 7-benzyltheophylline. Many N-7 or N-9 alkylpurines undergo attack at C-8 by hydroxyl ions, with subsequent fission of the imidazole ring, a result which reflects their inability to assume the more stable anionic form. In order to test this possibility 7-benzyltheophylline was heated under reflux with an equimolar amount of sodium hydrogen carbonate solution for some hours. No evidence of a ring-opened derivative was obtained, only starting material in good yield was recovered. This result, therefore, virtually rules out the possibility that the "8-benzyltheophylline monohydrate" of Serchi contains any of the isomeric 5-(N-benzyl)formamidopyrimidine (8).

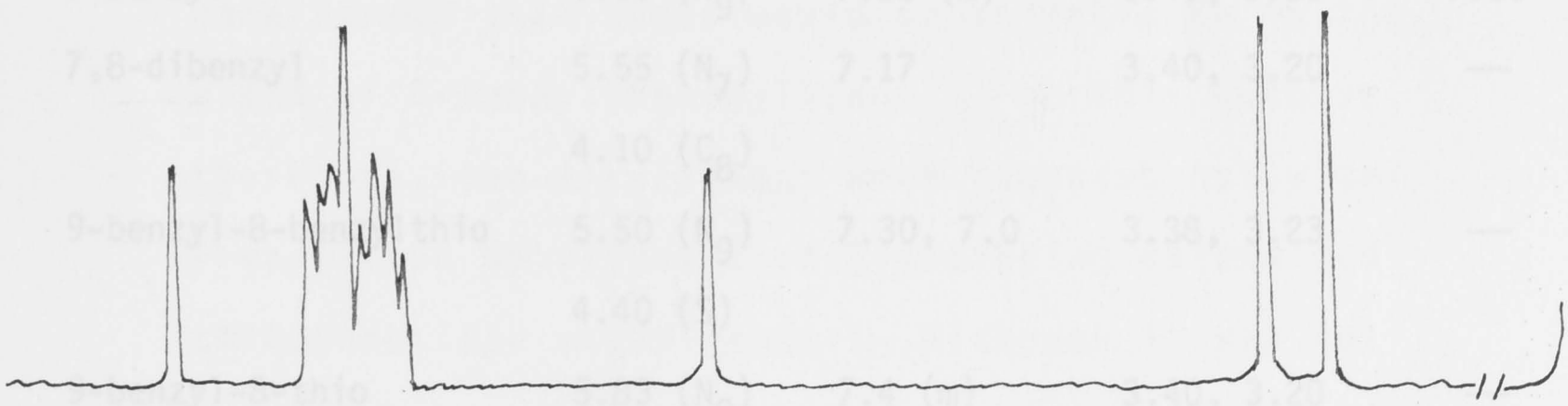
a. 7-benzyl



b. 8-benzyl



c. 9-benzyl



d. 7,8-dibenzyl

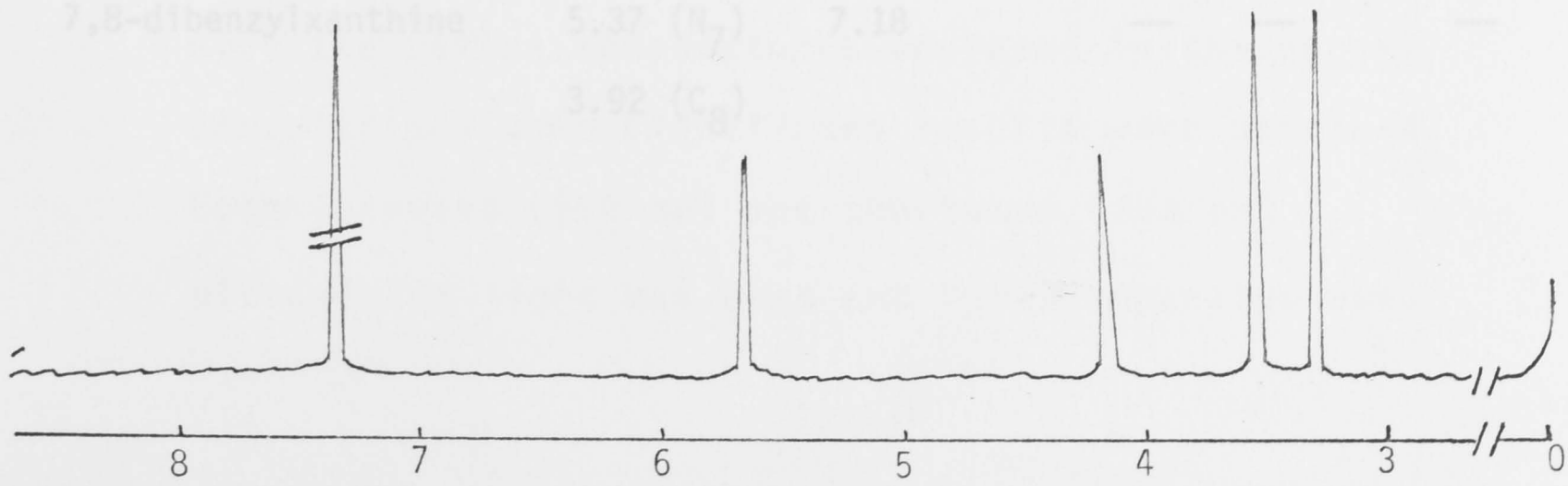
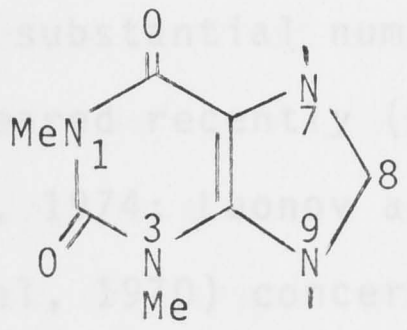


Figure 1

Table 1

^{1}H N.m.r. spectra of Theophylline derivatives

(δ values in DMSO, TMS as internal standard)



Substituent	CH_2 (benzyl)	C_6H_5	$Me(N_1)$, $Me(N_3)$	8-H
None	—	—	3.47, 3.25	8.05
7-benzyl	5.45 (N_7)	7.30	3.39, 3.19	8.27
8-benzyl	4.05 (C_8)	7.31	3.41, 3.23	—
9-benzyl	5.65 (N_9)	7.30 (m)	3.40, 3.20	7.83
7,8-dibenzyl	5.55 (N_7) 4.10 (C_8)	7.17	3.40, 3.20	—
9-benzyl-8-benzylthio	5.50 (N_9) 4.40 (S)	7.30, 7.0	3.38, 3.23	—
9-benzyl-8-thio	5.83 (N_9)	7.4 (m)	3.40, 3.20	—
8-phenyl	—	8.12, 7.48	3.50, 3.27	—
7,8-dibenzylxanthine	5.37 (N_7) 3.92 (C_8)	7.18	— —	—

b. Benzylation of theophylline under U.V. Irradiation

In the benzylation experiments described earlier in section (1Ba) theophylline is presumed to react in the anionic form and undergo attack by benzyl cations. Quite a substantial number of publications have, however, appeared recently (Christensen et al., 1975; Maeda et al., 1974; Leonov and Elad, 1974; Jerumanis and Martel, 1970) concerning the direct C-8 alkylation of purines under conditions which favour formation of alkyl radicals rather than cations.

In view of these findings, which demonstrated that alkyl radicals can be formed and react under very mild conditions, some preliminary benzylation experiments were conducted using procedures which promote benzyl radical formation. It was expected that if radicals were formed then there would be an increase in the yield of 8-benzyltheophylline. For this purpose, therefore, the alkylations were repeated but with irradiation by ultraviolet light being maintained throughout the experiments. Although it was not practical to attain the original benzylation conditions exactly, for example the highest temperature at which the irradiation could be carried out was 90^{o} compared with the reflux temperatures employed in the normal benzylation, some significant results were obtained. Both longwave (350 nm) and shortwave (254 nm) ultraviolet light was used and Pyrex apparatus was

extensively employed. This type of glassware is reported to be impervious to shortwave u.v. light below 310 nm and to overcome this difficulty some parallel reactions were carried out using the longer wavelength (355 nm) u.v. radiation. It was, therefore, interesting to compare the results obtained with the two different radiation sources with those from the experiments performed in visible light. The results given in Table 2, show the weight of products isolated using an initial weight of 5 g of theophylline for each reaction; this was converted into the soluble sodium salt either before or during the reaction.

Before comparing the results of the visible light and u.v. light procedures account must be taken of the amount of starting material which remains at the end of each experiment. Whereas only a small quantity (25-30%) is recovered under the normal alkylating conditions this figure approximates to 50% when irradiation is employed. Thus, the yields of benzylated derivatives must be viewed against the actual amount of theophylline consumed in the reaction. On the basis of these facts then it can be demonstrated that the 0.5% overall yield of the 7,8-dibenzylpurine formed in visible light (100^{o}/4h) rose to 1.2% (90^{o}/12h) in the presence of 350 nm light. However, in shorter wavelength light, little or none of this derivative can

be detected. More interesting results are obtained for the formation of 8-benzyltheophylline where the visible light value of 0.8% (74^{o}/12h) rises nearly twentyfold to a significant 14% under the same conditions but in the presence of 254 nm radiation. In this case time and temperature appear to have a degradative effect as the yield of this isomer drops to half of the above figure when the irradiation proceeds for 24 h at 90^{o}. It is of interest to find that along with the increase in the 8-benzylpurine under irradiation there is a corresponding decrease in the normal alkylation product, 7-benzyltheophylline. This falls from the visible light value of 36% (74^{o}/12h) down to 26% using short wavelength treatment with the same time and temperature. If these conditions are intensified or prolonged, then, as in the case of the 8-benzyl isomer, i.e. at 90^{o}/24h, the yield drops further to about one half of this figure.

The most significant feature that is noted from these results, when viewed overall, is the marked increase in the yield of 8-benzyltheophylline under irradiation, together with the reduction, to a lesser extent, in the formation of the normal 7-alkylated product. In view of the tendency of radicals for C- rather than N-substitution these findings lend support to the idea of a radical rather

Table 2

Benzylation of theophylline (5 g) with benzyl halide in aqueous alkali

Radiation	Reaction conditions	Products			Unreacted theophylline
		7-benzyl	8-benzyl	7,8-dibenzyl	
Visible	21°, 12h	0.01 g*	trace*	—	0.7*
	74°, 12h	2.3	0.05	—	0.7
	100°, 4h	2.5	0.08	0.04	0.5
U.v.(350 nm)	90°, 12h	1.0	0.15	0.06	2.5
U.v.(254 nm)	74°, 12h	0.8	0.50	—	2.4
	90°, 6h	0.7	0.26	possible trace	2.4
	90°, 24h	0.4	0.14	possible trace	2.3
	74°, 12h	—	—	—	4.0†

* Yields from 1 g theophylline only.

† theophylline, as neutral molecule, in 50% aqueous acetone.

ionic mechanism operating in the formation of the 8-benzyl derivative.

It was expected, in view of the isolation of some 7,8-dibenzyltheophylline during the original (visible light) benzylation procedures, that under the u.v. light conditions this yield would be significantly increased. In fact, this did appear to occur to some extent with the long wavelength u.v. light but not at all, or possibly only in trace amounts, with the shorter wavelength light.

No clear cut rationale can be advanced to explain the production of the 7,8-dibenzyl homologue. The results from the visible light experiments suggest that in the formation of this derivative a temperature effect operates; this is also discussed in a later section (1Ca). Thus, after benzylation for 12 h at 74^{o} none of the dialkylated purine can be detected whereas some is found to be present after 4 h at 100^{o}. Therefore, the relatively small increase in yield following long wavelength u.v. irradiation may be due only to a time rather than a photochemical effect. It would be reasonable then, in view of these findings, to suggest that initial formation of the 8-benzyl derivatives takes place by a radical attack, this purine then undergoing a much slower ionic benzylation at N-7 because of the moderate temperatures involved (see Section 1Ca). A fact which still awaits

clarification is the absence of any dibenzylated product when the short wave light is employed even though these conditions have given the highest yields of the 8-benzyl derivative.

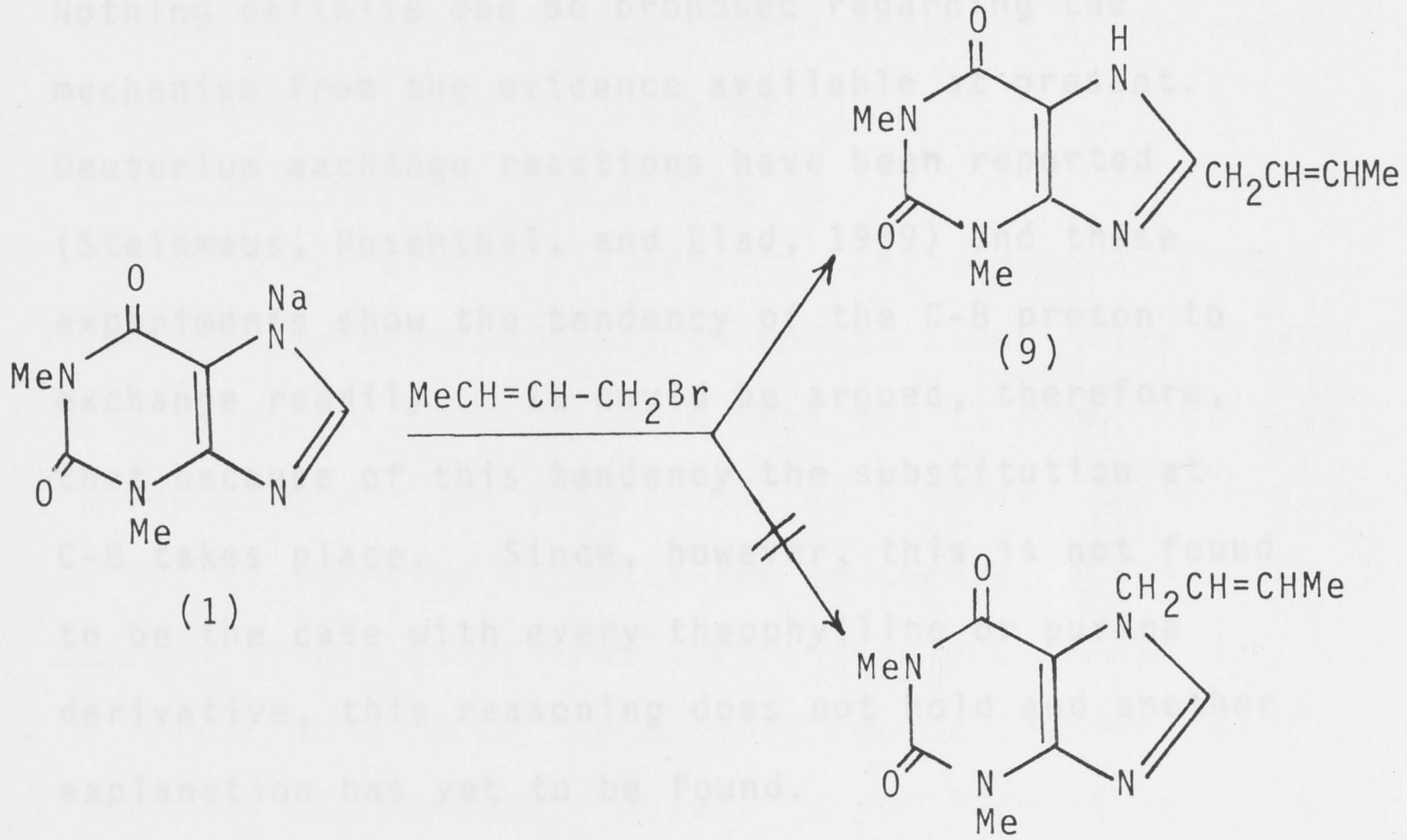
Notable also was the failure of 7-benzyl-theophylline to undergo benzylation at C-8 when treated with benzyl bromide in sodium hydrogen carbonate solution (74^{o}/12h) under short wavelength irradiation. It seems, therefore, that similar considerations to those given for the benzylation of 8-benzyltheophylline at N-7 could apply. Thus, because of steric hindrance due to the benzyl group already present, more severe reaction conditions than those used are needed to allow the second benzyl group to be inserted.

The gradual decrease in the yield of both 7- and 8-benzyltheophylline that is found to occur with prolonged exposure to short wavelength light at elevated temperature is presumed to be due to photolytic degradation. Examples of fairly rapid breakdown of xanthine derivatives in solution are recorded (Matsuura and Saito, 1967) following exposure of them to ultraviolet radiation.

c. Action of crotyl bromide on theophylline

An unusual but nevertheless authentic case of direct C-8 alkylation, reported by Donat et al. (1959), arises when sodium theophyllinate is treated with crotyl bromide at room temperature (Scheme 4) giving 8-crotyltheophylline (9) as the sole product. It is surprising to find the absence of any N-7 alkylated product (10) in view of the ease with which theophylline usually gives the 7-alkyl derivatives under such conditions.

This second example reported of unexpected reactivity of the 8-position in theophylline prompted also the re-examination of this alkylation as an extension of the previous benzylation studies. When this experiment was repeated, under the conditions originally employed a white precipitate was obtained. On crystallisation, a sample of this gave 8-crotyl-theophylline; the m.p. and n.m.r., of which were in agreement with those of the authentic derivative which had been obtained by an unambiguous synthesis (Donat, 1959). In order to find out if there was any N-7 alkylated theophylline present the precipitate was digested with alkali. However, no trace of the insoluble N-7 alkyl derivative could be found.



(1)

(9)

(10)

Scheme 4

This was further confirmed by t.l.c. examination. These results, therefore, confirmed the original workers' findings.

The formation of only 8-crotyltheophylline from the reaction of theophylline with crotyl bromide under remarkably mild conditions is abnormal. Nothing definite can be proposed regarding the mechanism from the evidence available at present. Deuterium exchange reactions have been reported (Steinmaus, Rosenthal, and Elad, 1969) and these experiments show the tendency of the C-8 proton to exchange readily. It could be argued, therefore, that because of this tendency the substitution at C-8 takes place. Since, however, this is not found to be the case with every theophylline or purine derivative, this reasoning does not hold and another explanation has yet to be found.

C. ATTEMPTED SYNTHESIS OF 7,8-DIBENZYLTHEOPHYLLINE

After having shown almost conclusively that the dibenzylated product, obtained during the benzylation of theophylline, was 7,8-dibenzyltheophylline, it remained at this juncture of the work to provide confirmatory proof by carrying out an unambiguous synthesis. During the various attempts made at this, which are described below, quite a number of interesting reactions were encountered.

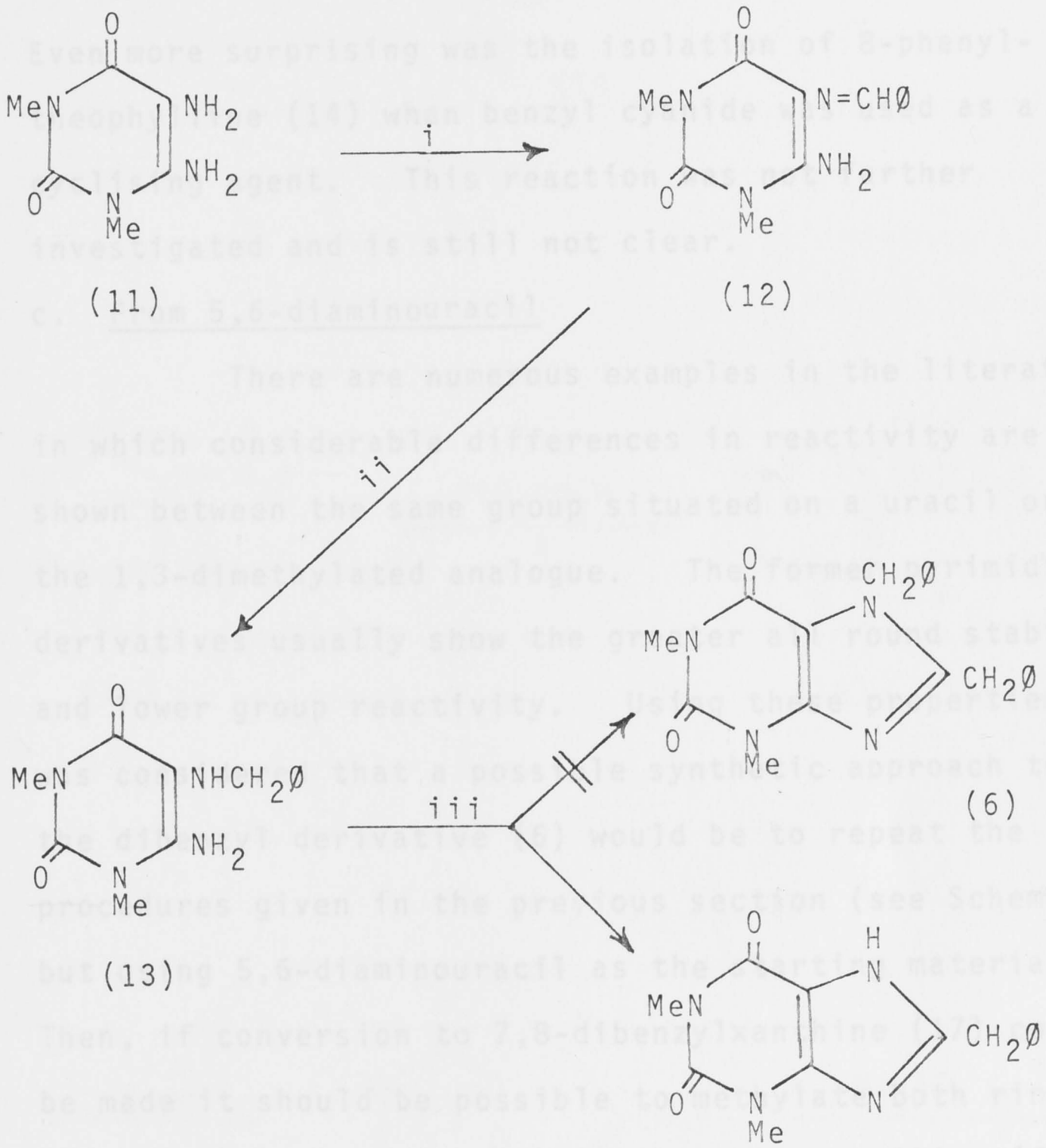
a. From 8-benzyltheophylline

Initially the benzylation of 8-benzyltheophylline in aqueous alkaline solution was tried under the same conditions as were employed to form 7-benzyltheophylline. At the end of the reaction, however, only the starting material was recovered. At this stage, it was considered that this might be due to steric hindrance by the 8-benzyl group towards the approach of the benzyl moiety at the 7-position. It was thought, therefore, that more forcing conditions should be tried to overcome any such steric effect. For this purpose the benzylation of the sodium salt of 8-benzyltheophylline was attempted with benzyl chloride in dimethylformamide under reflux conditions. Upon completion of the reaction the solid product obtained was found to have the same m.p., i.r., n.m.r., t.l.c. and analysis as the dibenzylated product obtained

from the benzylation of theophylline. Since, as has been noted already, derivatives of theophylline under alkaline alkylating conditions give only N-7 alkyl derivatives, it was concluded that this product was 7,8-dibenzyltheophylline. This benzylation result therefore, confirms that one of the benzyl groups is occupying the 8-position in the dibenzylated product obtained during the benzylation of theophylline. However, this alkylation product has not been derived from a truly ambiguous synthesis and other routes were therefore sought to achieve this.

b. <u>From 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione</u>

This approach involved initially the formation of a 6-amino-5-benzylaminopyrimidine which it was then hoped could be cyclised to the required purine using Traube type conditions (Scheme 5). The key starting material, 6-amino-5-benzylamino-1,3-dimethylpyrimidine-2,4-dione (13), was obtained with difficulty by catalytic reduction of the Schiff base (12) derived from 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione (11) and benzaldehyde (Traube and Nithack, 1906). A number of attempts were made to cyclise the intermediate pyrimidine (13) derivative, using either phenylacetic acid or phenyl acetamide as the cyclising agent, but in all cases the product obtained was 8-benzyltheophylline (3). This indicated unusual reactivity of the benzyl group and that a loss of a benzyl group had occurred either



i = ØCHO

ii = H_2/PtO_2

iii = $ØCH_2CO_2H$ or $ØCH_2CONH_2$

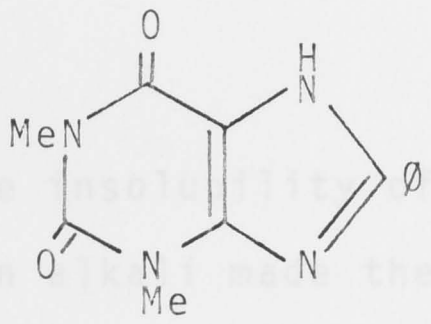
Scheme 5

from the 5-amino group in the pyrimidine (13) or if it had been formed, from the 7,8-dibenzylpurine (6). Even more surprising was the isolation of 8-phenyl-theophylline (14) when benzyl cyanide was used as a cyclising agent. This reaction was not further investigated and is still not clear.

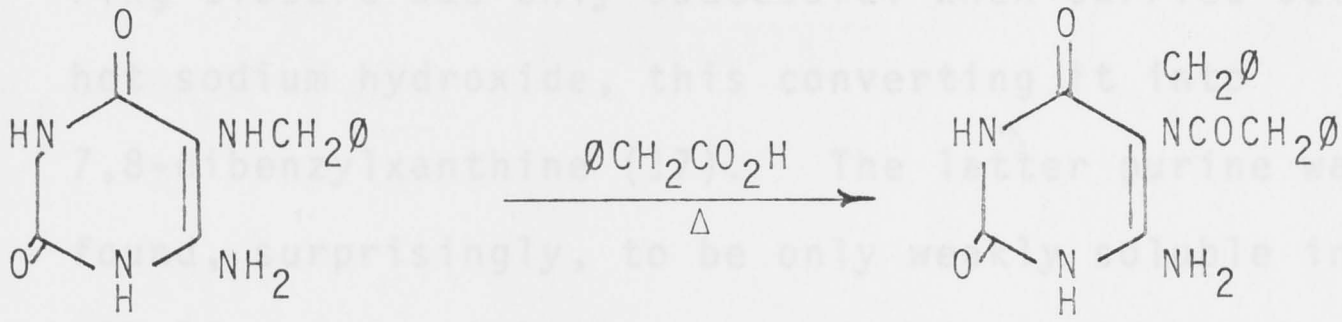
c. From 5,6-diaminouracil

There are numerous examples in the literature in which considerable differences in reactivity are shown between the same group situated on a uracil or the 1,3-dimethylated analogue. The former pyrimidine derivatives usually show the greater all round stability and lower group reactivity. Using these properties it was considered that a possible synthetic approach to the dibenzyl derivative (6) would be to repeat the procedures given in the previous section (see Scheme 5) but using 5,6-diaminouracil as the starting material. Then, if conversion to 7,8-dibenzylxanthine (17) could be made it should be possible to methylate both ring nitrogen atoms to give the required theophylline (6).

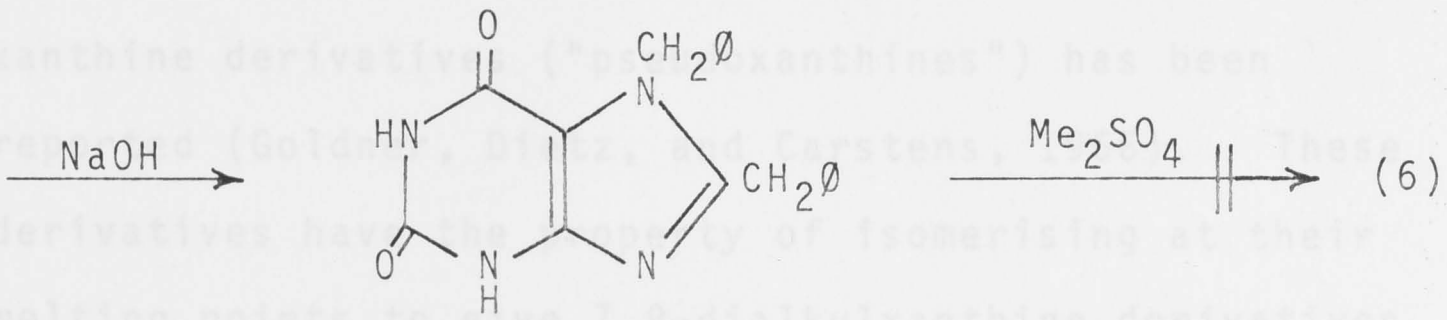
The intermediate 6-amino-5-benzylaminouracil (15) was obtained by the sodium amalgam reduction of the Schiff base derived from 5,6-diaminouracil and benzaldehyde using the method of Traube (1923). This gave superior yields of reduced compound compared with those obtained with the corresponding NN'-dimethylated homologue using catalytic hydrogenation (see previous section).



(14)



(15) (16)

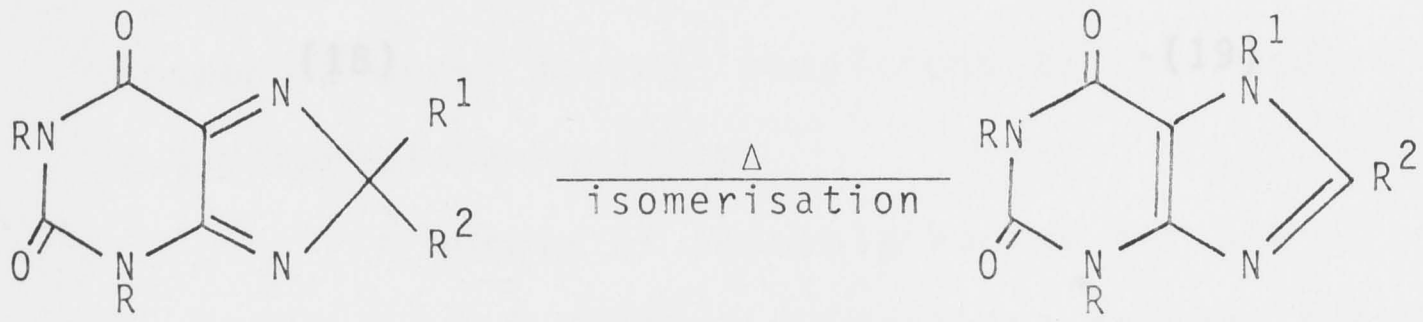


(17) (6)

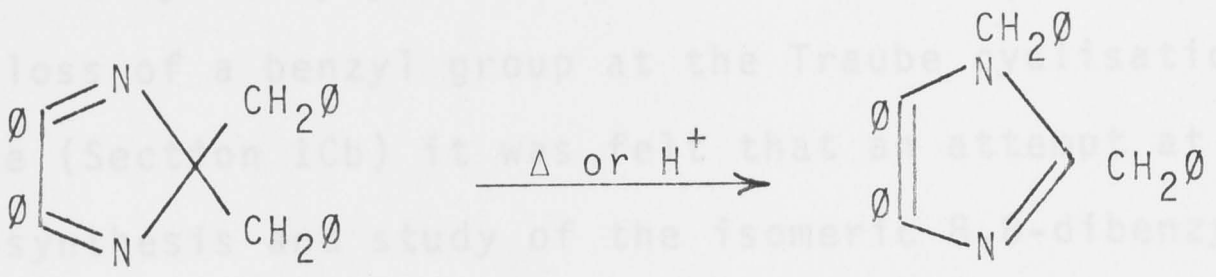
Unfortunately, the insolubility of the dimethyluracil derivative (12) in alkali made the amalgam reduction unsuitable in this case. The fusion of 6-amino-5-benzylaminouracil with phenylacetic acid readily gave 6-amino-5-(N-benzyl)phenylacetamidopyrimidine-2,4-dione (16). Although various high boiling solvents were employed in attempts to cyclise this intermediate, the ring closure was only successful when carried out in hot sodium hydroxide, this converting it into 7,8-dibenzylxanthine (17). The latter purine was found, surprisingly, to be only weakly soluble in alkali and possibly because of this could not be methylated under the normal conditions employed (methyl sulphate in sodium hydroxide) to the required 7,8-dibenzyltheophylline (6).

d. Through 8,8-dibenzyl-8H-xanthines

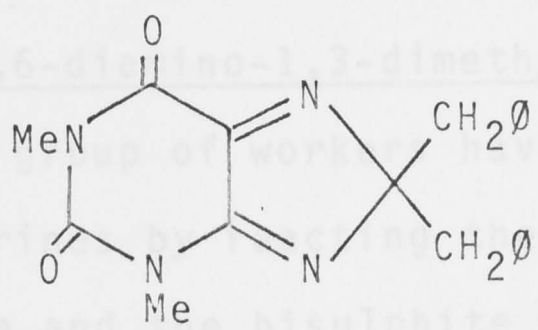
The synthesis of a number of 8,8-dialkyl-8H-xanthine derivatives ("pseudoxanthines") has been reported (Goldner, Dietz, and Carstens, 1966). These derivatives have the property of isomerising at their melting points to give 7,8-dialkylxanthine derivatives (Scheme 6). Such a rearrangement is not unexpected in view of a report some years' earlier by Weiss (1952) in which some 2,2-dialkylimidazoles, including the 2,2-dibenzyl-4,5-diphenylimidazole (18), rearranged to give the corresponding 1,2-dialkyl-4,5-diphenylimidazole, the dibenzyl derivative (18) forming the isomer (19) when heated or treated with acids.



Scheme 6



(18) (19)



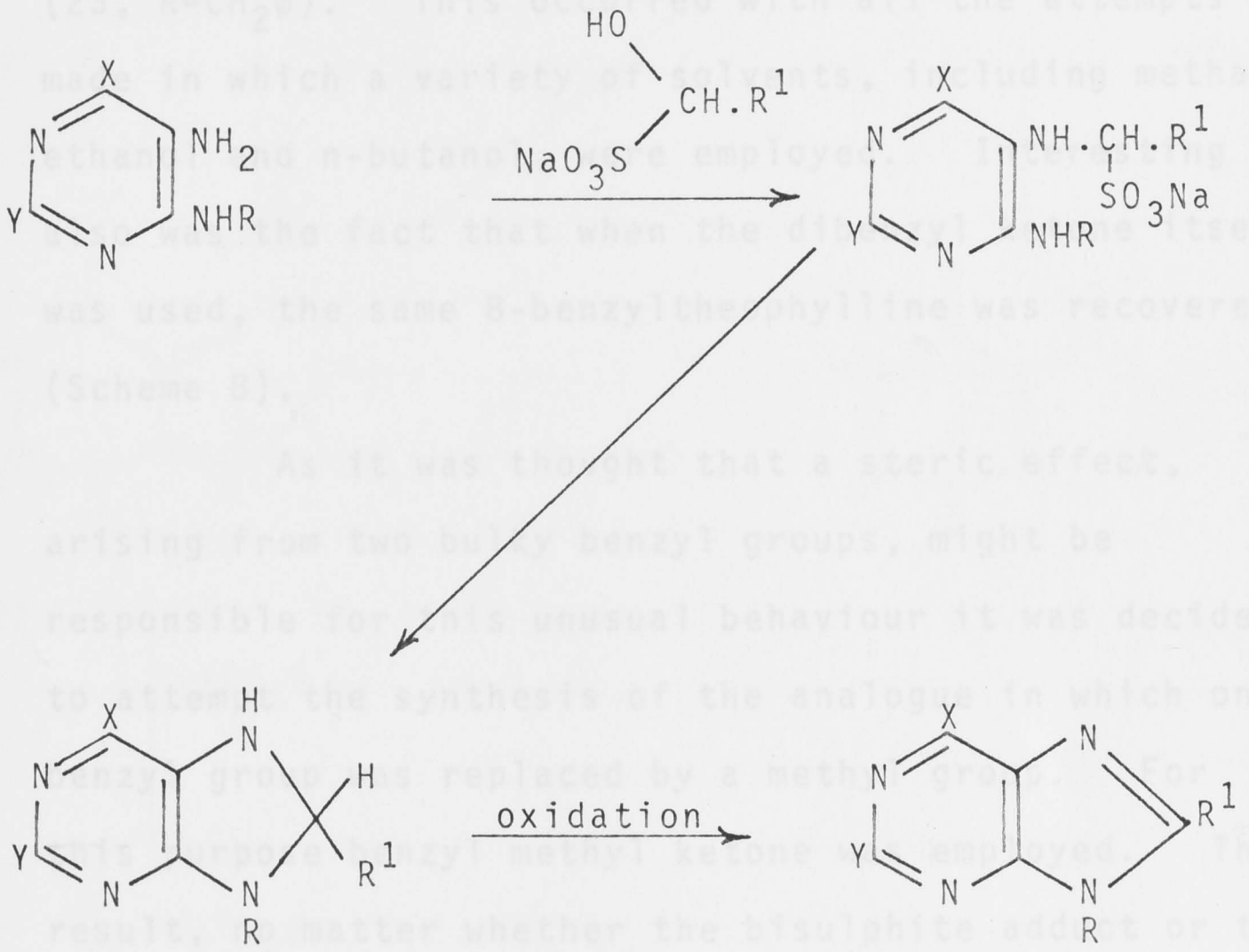
(20)

Since the main attempt to synthesise 7,8-dibenzyltheophylline directly had failed, due to the loss of a benzyl group at the Traube cyclisation stage (Section 1Cb) it was felt that an attempt at the synthesis and study of the isomeric 8,8-dibenzyl-8H-xanthine might be of use, as this derivative should isomerise under thermal conditions to the required 7,8-dibenzyltheophylline.

A number of possible routes for the syntheses of 8,8-dialkyl-8H-xanthine derivatives were contemplated, those attempted are described below.

From 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione — One group of workers have synthesised 8-substituted purines by reacting the appropriate diaminopyrimidine and the bisulphite product of the aldehyde (Ridley, Spickett, and Timmis, 1965). The general mechanism proposed by these workers for the reaction, shown in Scheme 7, involved an intermediate 7H,8H-dihydropurine which then underwent subsequent in situ oxidation to the purine.

It seemed worthwhile therefore, to try the condensation of 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione and the bisulphite product of dibenzyl ketone in the hope of obtaining the required 8,8-dibenzyl-8H-xanthine (20). This it was expected would be formed through oxidation of the initially formed

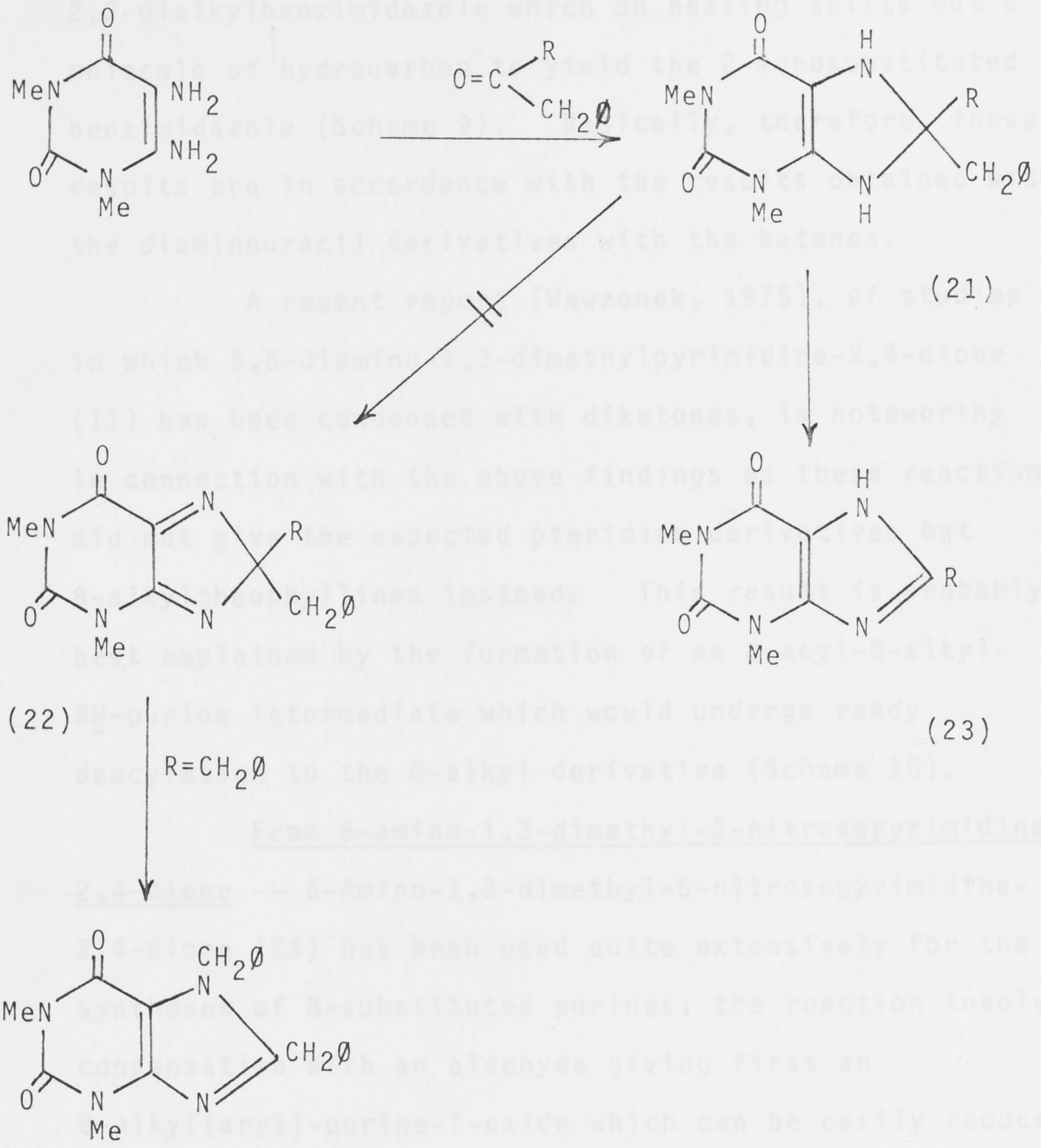


Scheme 7

dihydro analogue (21). The product actually isolated from the reaction was, in fact, neither the 8,8-dibenzylpurine (22, R=$CH_2\emptyset$) nor the 7,8-dibenzylated isomerisation product (6) but 8-benzyltheophylline (23, R=$CH_2\emptyset$). This occurred with all the attempts made in which a variety of solvents, including methanol, ethanol and n-butanol, were employed. Interesting also was the fact that when the dibenzyl ketone itself was used, the same 8-benzyltheophylline was recovered (Scheme 8).

As it was thought that a steric effect, arising from two bulky benzyl groups, might be responsible for this unusual behaviour it was decided to attempt the synthesis of the analogue in which one benzyl group was replaced by a methyl group. For this purpose benzyl methyl ketone was employed. The result, no matter whether the bisulphite adduct or the ketone itself was used, was the same, only 8-methyl-theophylline (23, R=Me) was isolated (Scheme 8). The identity of this product was checked by carrying out an unambiguous synthesis.

These results, which seemed at first to be quite unusual can be compared with those, reported by Elderfield and Meyer (1954) for the reaction of o-phenylenediamine with dialkylketones. Initially, the elimination of water occurs to give the intermediate

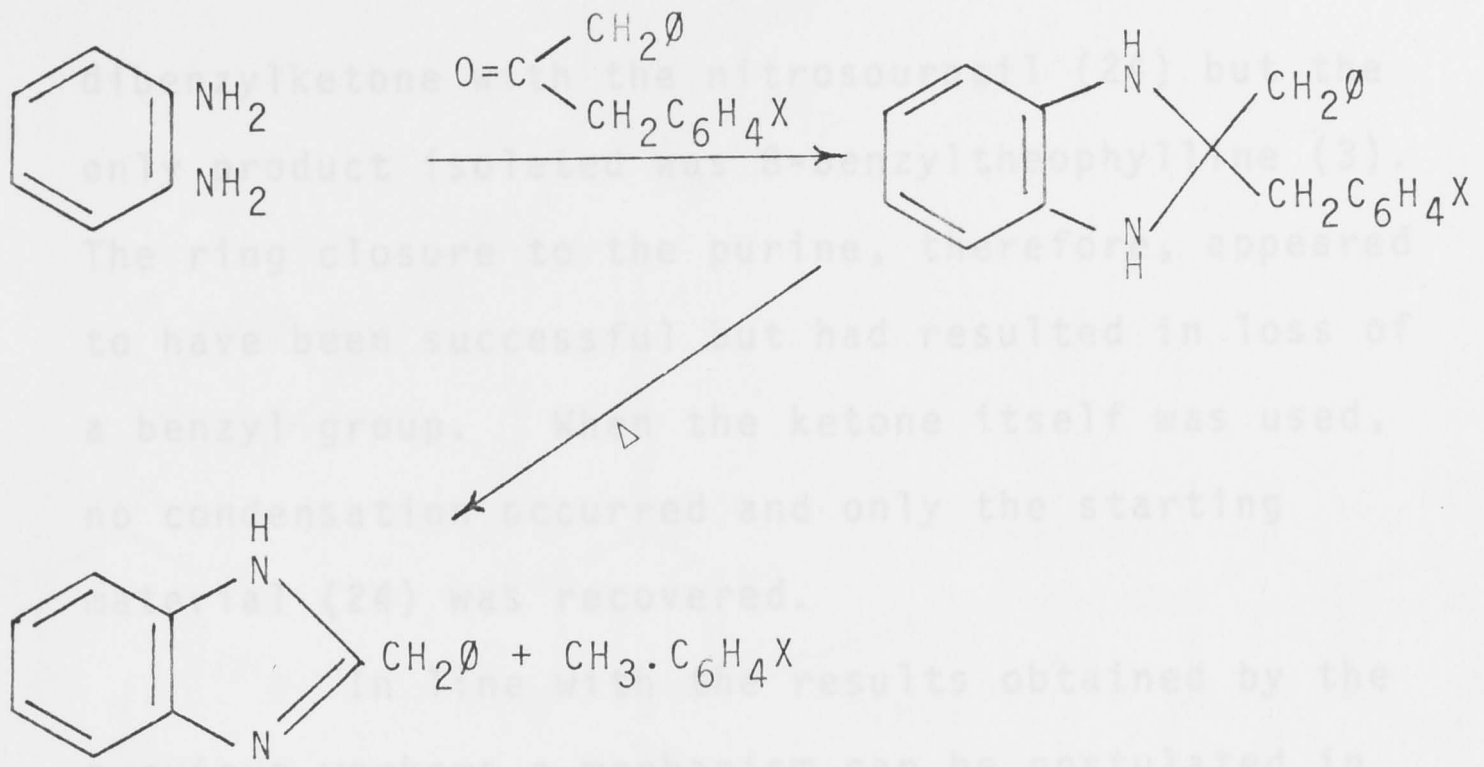


Scheme 8

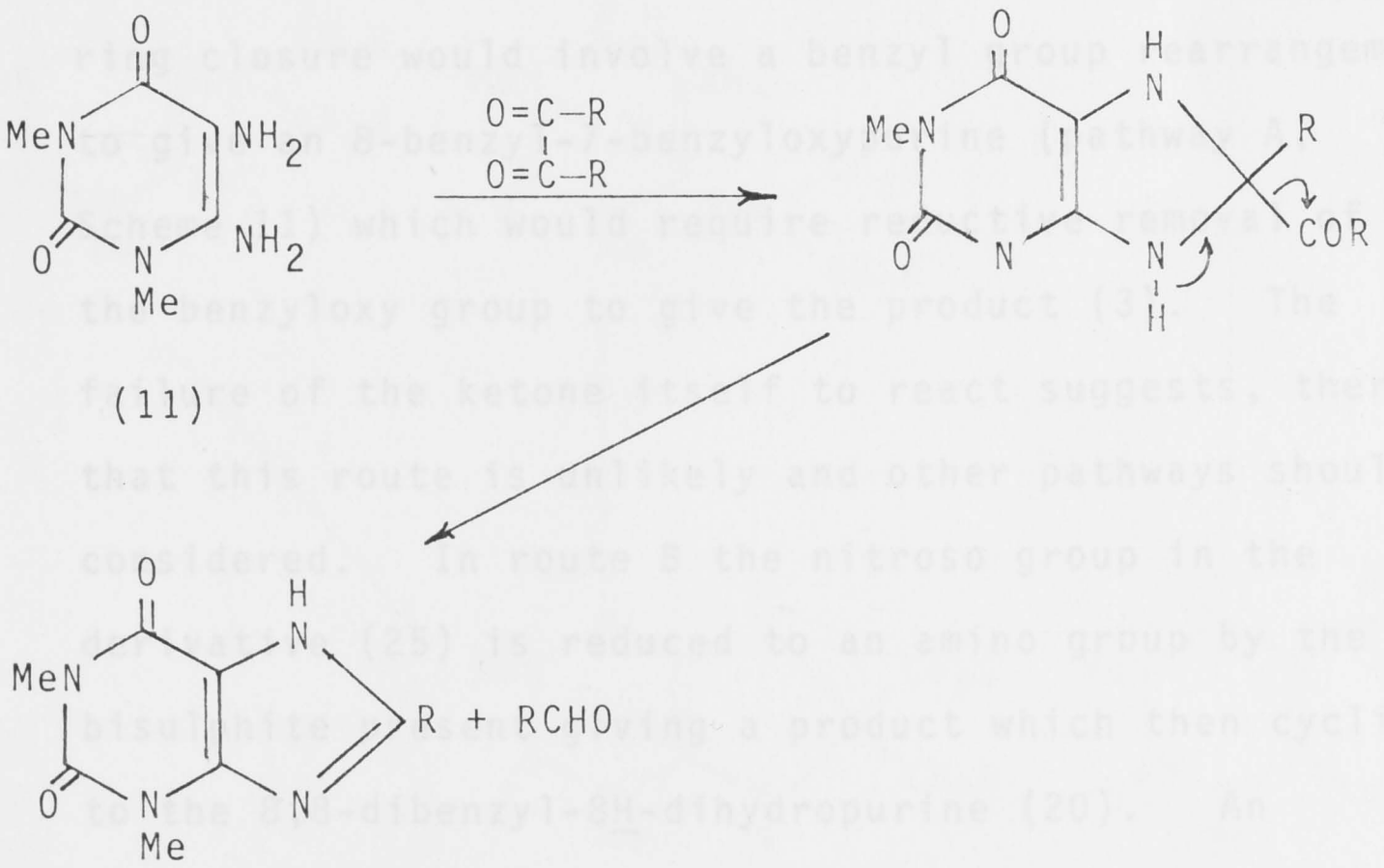
2,2-dialkylbenzimidazole which on heating splits out a molecule of hydrocarbon to yield the 2-monosubstituted benzimidazole (Scheme 9). Basically, therefore, these results are in accordance with the results obtained with the diaminouracil derivatives with the ketones.

A recent report (Wawzonek, 1976), of studies in which 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione (11) has been condensed with diketones, is noteworthy in connection with the above findings as these reactions did not give the expected pteridine derivatives but 8-alkyltheophyllines instead. This result is probably best explained by the formation of an 8-acyl-8-alkyl-8H-purine intermediate which would undergo ready deacylation to the 8-alkyl derivative (Scheme 10).

From 6-amino-1,3-dimethyl-5-nitrosopyrimidine-2,4-dione — 6-Amino-1,3-dimethyl-5-nitrosopyrimidine-2,4-dione (24) has been used quite extensively for the syntheses of 8-substituted purines; the reaction involves condensation with an aldehyde giving first an 8-alkyl(aryl)-purine-7-oxide which can be easily reduced to the purine (Goldner, Dietz, and Carstens, 1966a; Taylor and Garcia, 1964). Taylor (1964) had isolated 8-phenyltheophylline from a condensation between 6-amino-1,3-dimethyl-5-nitrosopyrimidine-2,4-dione (24) and benzaldehyde in dimethylformamide. This procedure was, therefore, tried using the bisulphite product of



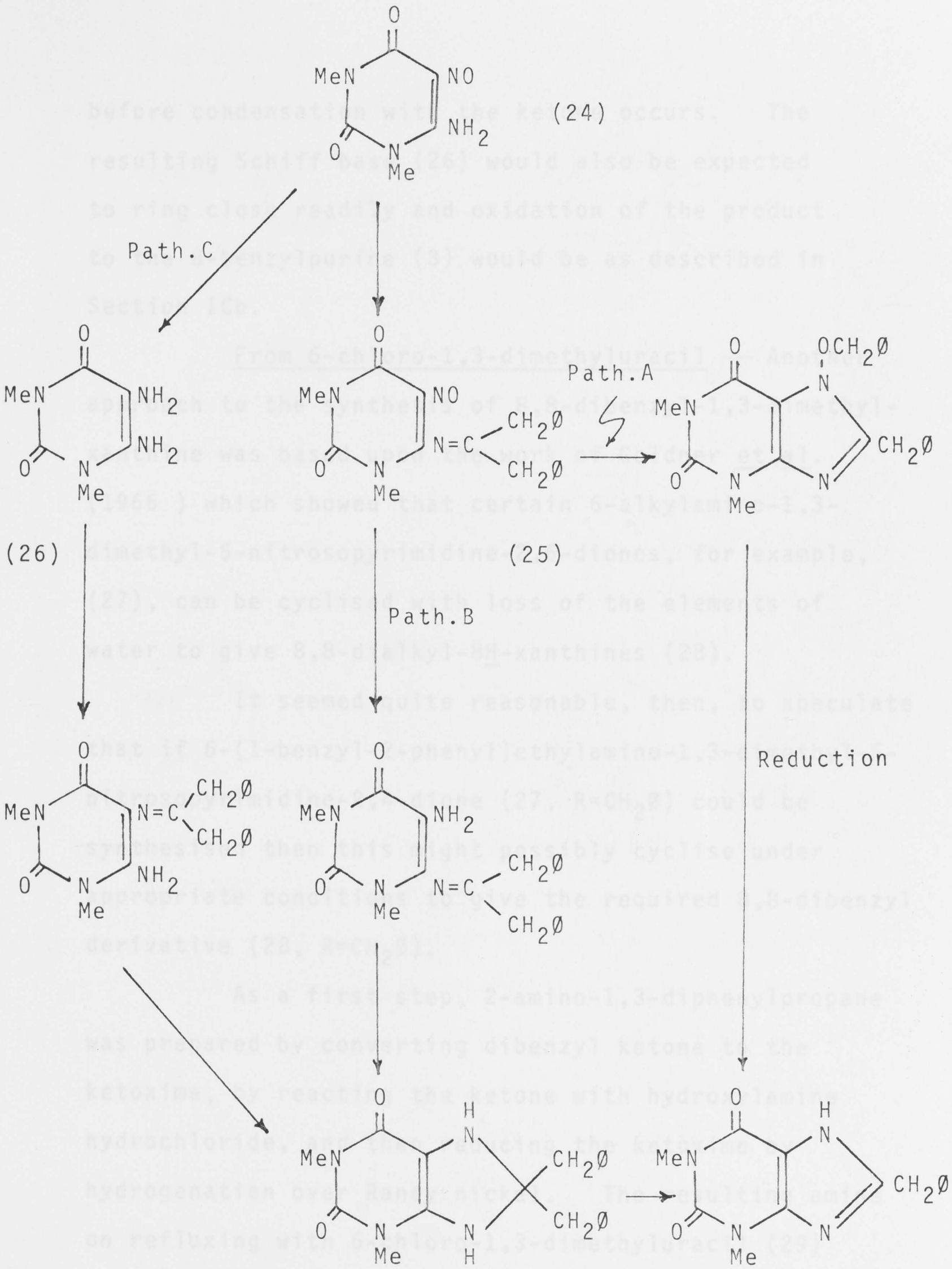
Scheme 9



Scheme 10

dibenzylketone with the nitrosouracil (24) but the only product isolated was 8-benzyltheophylline (3). The ring closure to the purine, therefore, appeared to have been successful but had resulted in loss of a benzyl group. When the ketone itself was used, no condensation occurred and only the starting material (24) was recovered.

In line with the results obtained by the previous workers a mechanism can be postulated in which as a first step an intermediate of the type (25) is formed. Such derivatives have been proposed by Goldner (1966a) and Taylor (1964) as the intermediates prior to cyclisation to the 7-hydroxypurines. In the case of the nitrosopyrimidine (25) an analogous ring closure would involve a benzyl group rearrangement to give an 8-benzyl-7-benzyloxypurine (pathway A, Scheme 11) which would require reductive removal of the benzyloxy group to give the product (3). The failure of the ketone itself to react suggests, therefore, that this route is unlikely and other pathways should be considered. In route B the nitroso group in the derivative (25) is reduced to an amino group by the bisulphite present giving a product which then cyclises to the 8,8-dibenzyl-8H-dihydropurine (20). An alternative is pathway C to this dihydropurine, by reduction of the nitroso group in the pyrimidine (24)



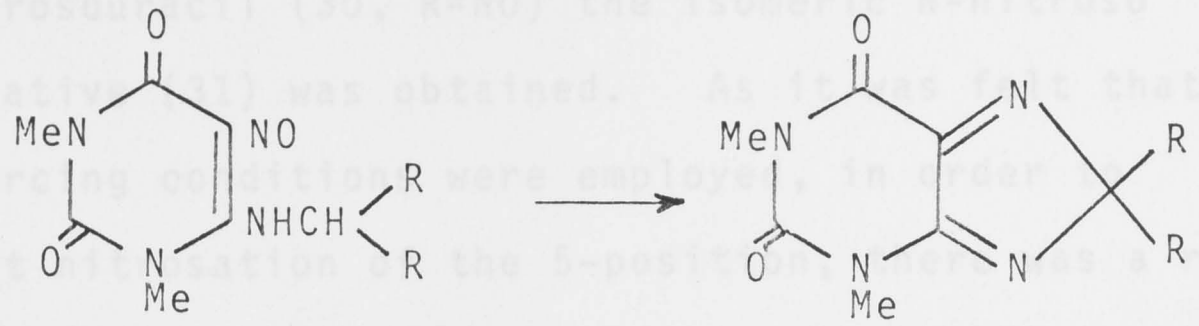
Scheme 11

before condensation with the ketone occurs. The resulting Schiff base (26) would also be expected to ring close readily and oxidation of the product to the 8-benzylpurine (3) would be as described in Section 1Cb.

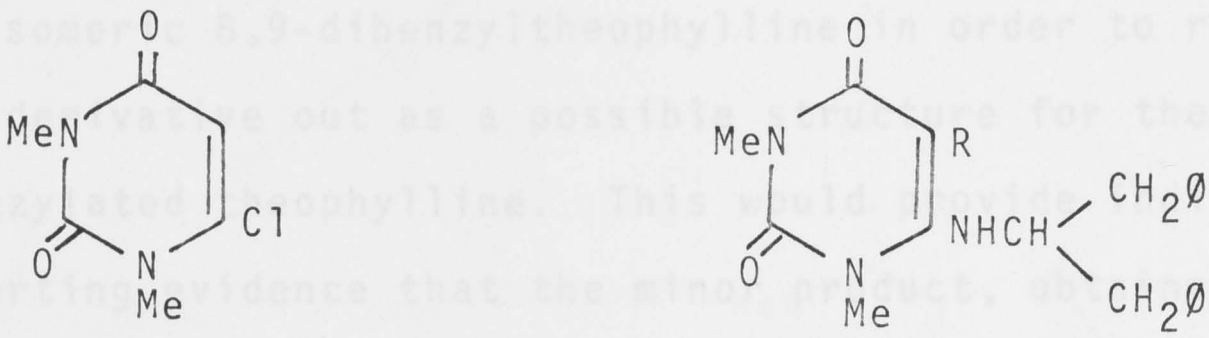
From 6-chloro-1,3-dimethyluracil — Another approach to the synthesis of 8,8-dibenzyl-1,3-dimethylxanthine was based upon the work of Goldner et al. (1966) which showed that certain 6-alkylamino-1,3-dimethyl-5-nitrosopyrimidine-2,6-diones, for example, (27), can be cyclised with loss of the elements of water to give 8,8-dialkyl-8H-xanthines (28).

It seemed quite reasonable, then, to speculate that if 6-(1-benzyl-2-phenyl)ethylamino-1,3-dimethyl-5-nitrosopyrimidine-2,4-dione (27, R=$CH_2Ø$) could be synthesised then this might possibly cyclise under appropriate conditions to give the required 8,8-dibenzyl derivative (28, R=$CH_2Ø$).

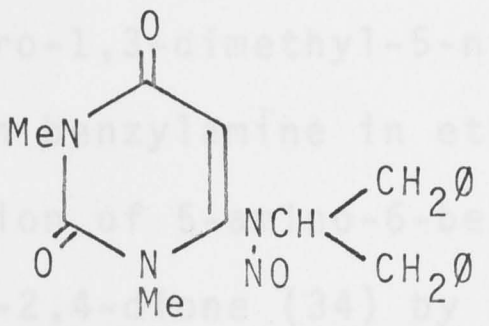
As a first step, 2-amino-1,3-diphenylpropane was prepared by converting dibenzyl ketone to the ketoxime, by reacting the ketone with hydroxylamine hydrochloride, and then reducing the ketoxime by hydrogenation over Raney nickel. The resulting amine on refluxing with 6-chloro-1,3-dimethyluracil (29) gave 6-(1-benzyl-2-phenyl)ethylamino-1,3-dimethylpyrimidine-2,4-dione (30, R=H). Unfortunately on nitrosation of this derivative, instead of the required



(27) (28)



(29) (30)



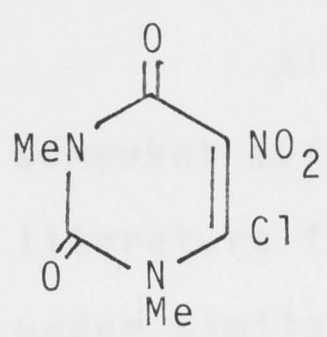
(31)

5-nitrosouracil (30, R=NO) the isomeric N-nitroso derivative (31) was obtained. As it was felt that if forcing conditions were employed, in order to effect nitrosation of the 5-position, there was a risk of removal of one of the benzyl groups, no further attempts at synthesis by this approach were made.

D. ATTEMPTED SYNTHESIS OF 8,9-DIBENZYLTHEOPHYLLINE

So far, having been unsuccessful in achieving an unambiguous synthesis of 7,8-dibenzyltheophylline, an alternative course was to attempt the synthesis of the isomeric 8,9-dibenzyltheophylline in order to rule this derivative out as a possible structure for the dibenzylated theophylline. This would provide indirect supporting evidence that the minor product, obtained during the benzylation of theophylline, was 7,8-dibenzyltheophylline (6).

The starting material for the proposed synthesis was 6-benzylamino-1,3-dimethyl-5-nitropyrimidine-2,4-dione (33); this was obtained quite easily by replacing the chlorine of 6-chloro-1,3-dimethyl-5-nitropyrimidine-2,4-dione (32) with benzylamine in ethanol. However, while the preparation of 5-amino-6-benzylamino-1,3-dimethylpyrimidine-2,4-dione (34) by catalytic reduction appeared to take place readily, with uptakes of hydrogen of the right order for reduction of the nitro group,



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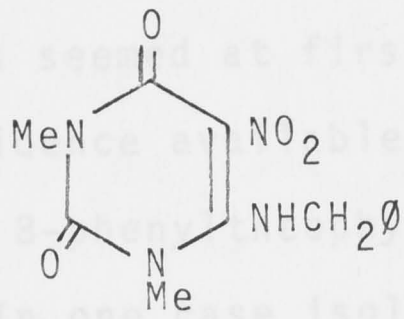

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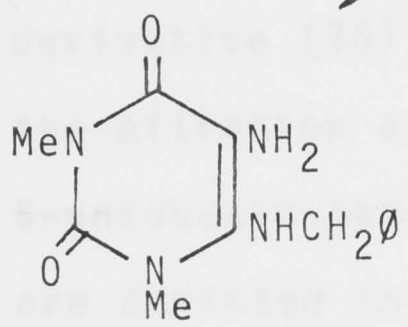
H_2/Ni or PtO_2



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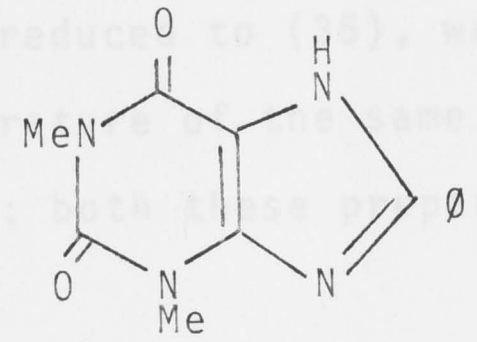
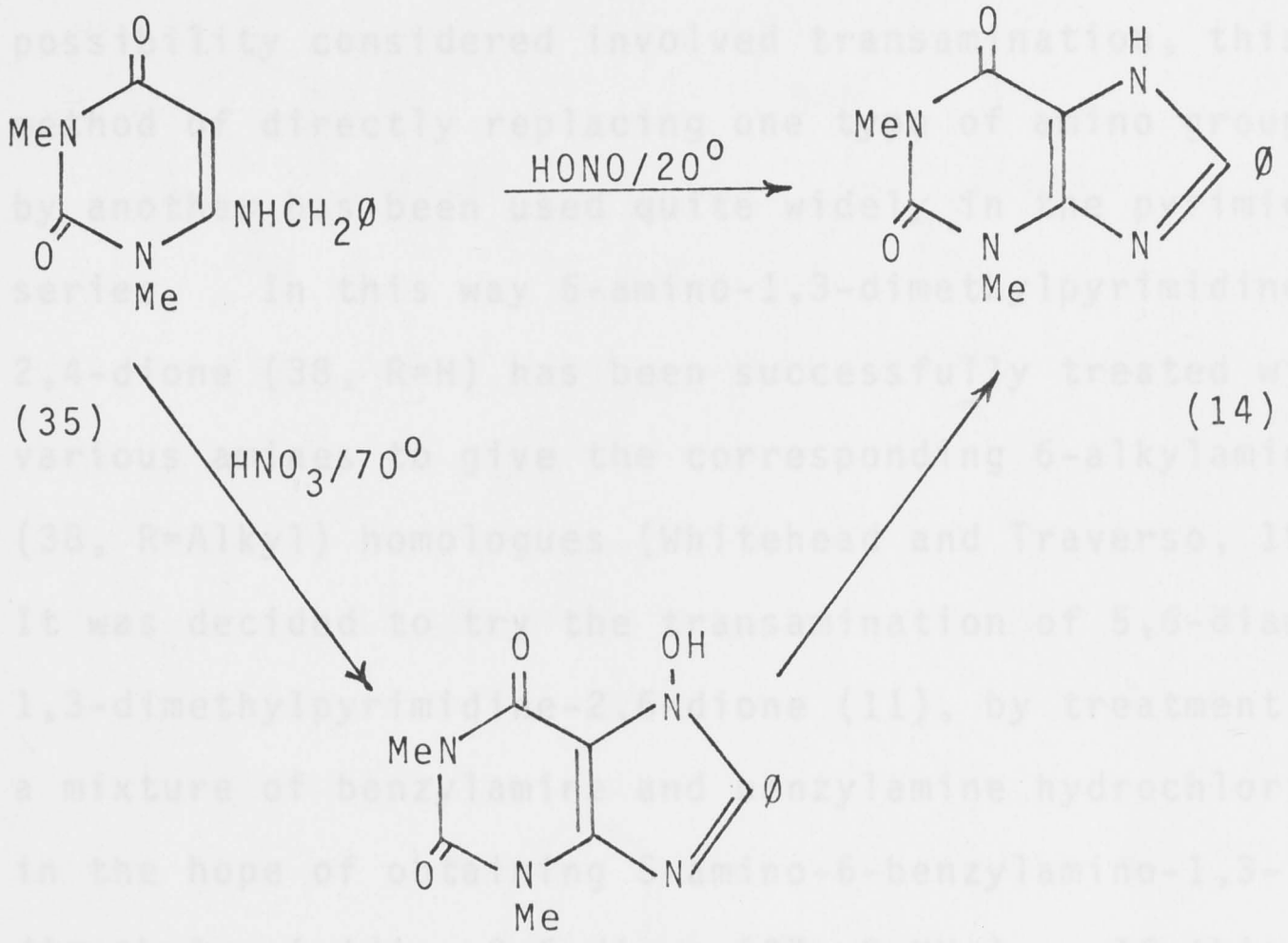


(14)

on working up the product was found to be 8-phenyl-theophylline (14). This occurred no matter whether Raney nickel or platinum was used as catalyst and also when alkaline ferrous sulphate was employed, using the method of Brown (1954), as reducing agent.

Although these results seemed at first somewhat surprising there is evidence available in the literature for the formation of 8-phenyltheophylline under similar circumstances. In one case isolation of this purine (14) is reported (Goldner, Dietz, and Carstens, 1966a) when nitrosation of the 6-benzylamino-5-unsubstituted-pyrimidine (35) was carried out in the cold. The other case which gave rise first to the derivative (36) which was then reduced to (35), was the nitration at moderate temperature of the same 5-unsubstituted-pyrimidine (35); both these preparations are depicted in Scheme 12.

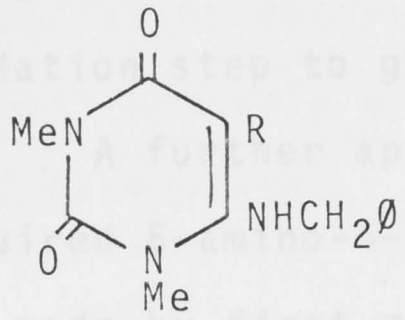
These two examples of purine formation, through cyclodehydration involving either a 5-nitroso or 5-nitro group, suggest an explanation for the formation of 8-phenyltheophylline during the catalytic reduction of the 5-nitro derivative (33). In converting the nitropyrimidine to the amino analogue the intermediate nitroso (37, R=NO) and hydroxyamino (37, R=NHOH) derivatives are involved. As both these contain a highly reactive group at the 5-position the cyclisation could occur readily at either stage.



Scheme 12

As the 5-amino-6-benzylaminopyrimidine (34) could not be obtained by reduction of the 5-nitro analogue a route using an intermediate having a 5-amino group already in place was sought. One possibility considered involved transamination, this method of directly replacing one type of amino group by another has been used quite widely in the pyrimidine series. In this way 6-amino-1,3-dimethylpyrimidine-2,4-dione (38, R=H) has been successfully treated with various amines to give the corresponding 6-alkylamino (38, R=Alkyl) homologues (Whitehead and Traverso, 1960). It was decided to try the transamination of 5,6-diamino-1,3-dimethylpyrimidine-2,6-dione (11), by treatment with a mixture of benzylamine and benzylamine hydrochloride, in the hope of obtaining 5-amino-6-benzylamino-1,3-dimethylpyrimidine-2,6-dione (37, R=NH_2). If this could be achieved then it should be possible to cyclise this derivative to the required 8,9-dibenzyltheophylline using either phenylacetic acid or phenylacetamide.

When the transamination was attempted the product obtained was, unexpectedly, 8-phenyltheophylline (14) instead of the required 5-amino-6-benzylamino-1,3-dimethylpyrimidine-2,6-dione (37, R=NH_2). While this reaction was not further investigated the most logical explanation would seem to be that, following transamination of the 6-amino group by the benzylamino group



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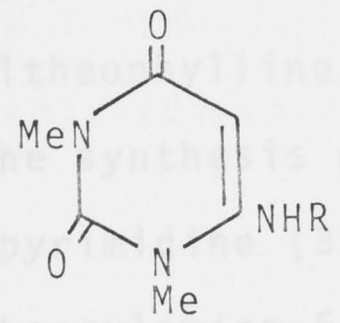

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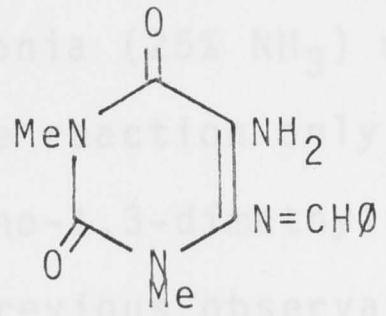


(39)

taking place, an oxidative ring-closure occurred under the thermal conditions of the procedure. One possible reaction sequence for this would be the formation first of the 5-amino-6-benzylideneaminopyrimidine (39), this being followed then by a ring-closure involving a second oxidation step to give 8-phenyltheophylline.

A further approach to the synthesis of the required 5-amino-6-benzylaminopyrimidine (37, R=NH_2) was made by first preparing 6-benzylamino-5-bromo-1,3-dimethylpyrimidine-2,6-dione (37, R=Br) from 6-benzylamino-1,3-dimethyluracil (37, R=H) by treatment with bromine (Bredereck, Herlinger, and Resemann, 1960). Subsequently a number of attempts were made to replace the bromo group by an amino group. Firstly, refluxing with aqueous ammonia (25% NH_3) was tried but after completion of the reaction only the debrominated product, i.e. 6-benzylamino-1,3-dimethyluracil (37, R=H) was obtained. One previous observation (Paul and Sen, 1963) of debrominations of this type occurring under similar conditions has been made in the pyrimidine series. When ethanolic ammonia instead was employed under reflux conditions, 8-phenyltheophylline (14) was obtained. In this case displacement of the bromine atom by a nitrogen containing group must have occurred at some stage prior to ring-closure but lack of evidence does not allow any conclusion to be drawn as to the mechanistic

pathway followed. As in the transamination experiments it seems more than likely that intermediate 5-amino-6-benzylaminopyrimidine is formed and this derivative undergoes an aerial oxidative cyclisation to the 8-phenylpurine, most probably by way of a benzylideneaminopyrimidine intermediate.

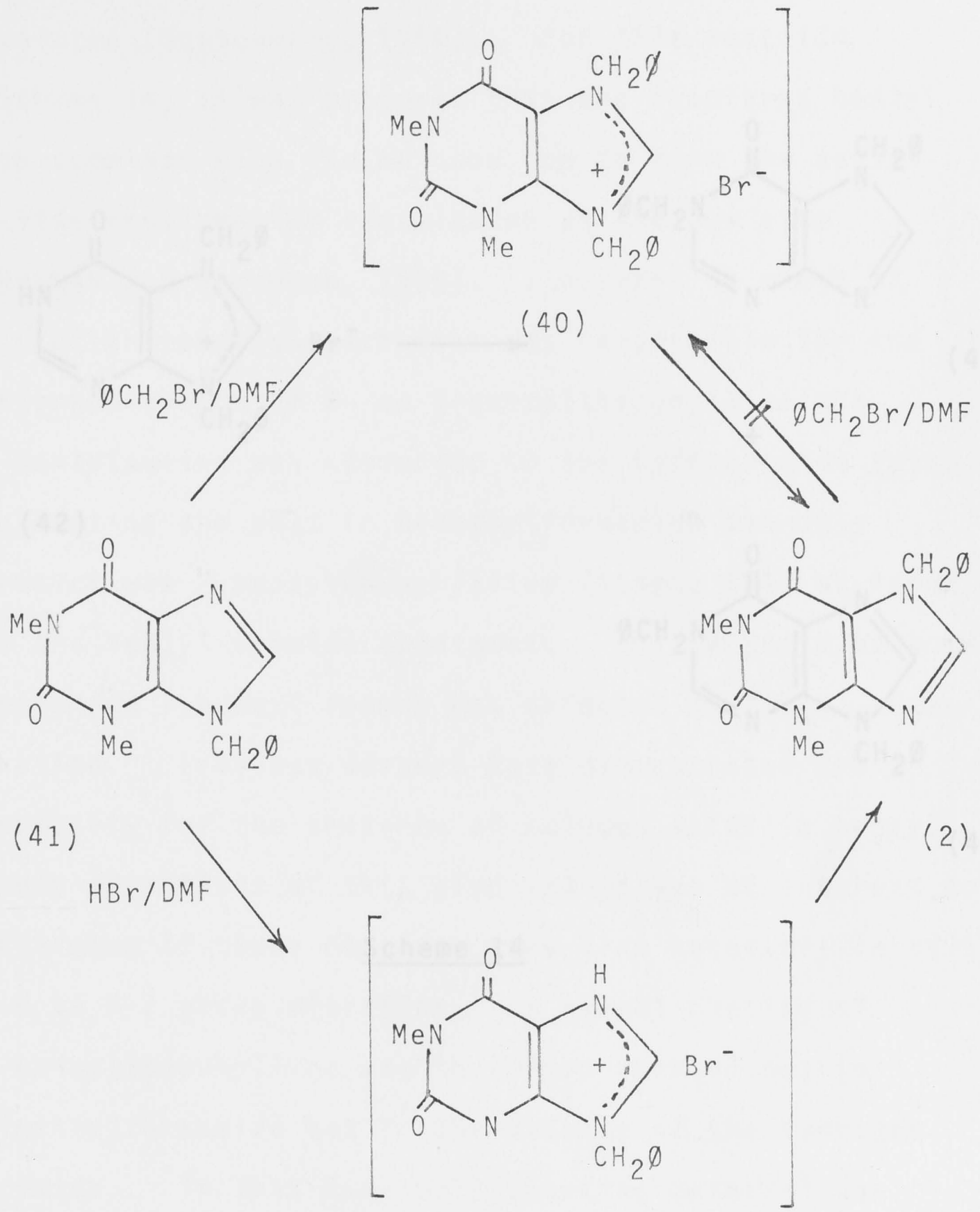
E. ATTEMPTED SYNTHESIS OF 7,9-DIBENZYLTHEOPHYLLINIUM BROMIDE

In addition to 7,8- and 8,9-dibenzyltheophylline a third dibenzyl derivative, the quaternary salt of 7,9-dibenzyltheophylline (40) was possible. Such a derivative was of interest because, firstly, it was expected that owing to the charged nature of the imidazole ring one of the benzyl groups might be labile and undergo rearrangement, which might include an N → C-8 migration, and secondly, to observe the effect of the chemical shifts due to location of the benzylic groups on the nitrogen atoms of the shared quaternary system. Some 7,9-dialkyl quaternary derivatives of xanthines of this type have been prepared under forcing conditions, using methyl p-toluenesulphonate at 170° (Bredereck, 1962). An attempt to quaternise 7-benzyltheophylline (2) was made therefore with benzyl bromide in dimethylformamide under reflux conditions (160°/30h). The product obtained on working up was apparently unchanged starting material (2). The benzylation was then repeated but using the same conditions as were employed in the benzylation of theophylline (Section 1Ba) i.e. in aqueous sodium hydrogen carbonate under reflux. Again, the 7-benzylpurine was recovered unchanged but t.l.c. examination of the concentrated mother liquor showed a spot corresponding to that of 7,8-dibenzyltheophylline. As

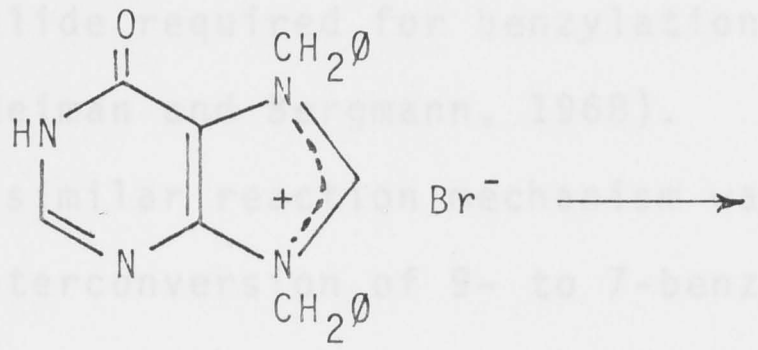
the amount present was not sufficient for isolation or characterisation of this derivative the presence of the spot cannot be taken as evidence of the formation of the 7,8-dibenzyl homologue.

In a fresh approach to forming the quaternary salt the benzylation of the 9-benzyl isomer (41) was considered; it was thought that in view of the fact that theophylline derivatives invariably underwent alkylation at N-7 it might be a more successful route. This purine was prepared by the method of Blicke and Schaaf (1956) the last stage of which involves the desulphurisation of the 9-benzyl-8-thiopurine (42) with nitrous acid using Biltz's (1921) procedure.

On heating 9-benzyltheophylline with benzyl bromide in dimethylformamide, under reflux, a different result was obtained from that above using the 7-benzyl isomer. In this case the main product was not starting material but 7-benzyltheophylline. The overall effect of this reaction was a migration of the benzyl group from N-9 to N-7 (Scheme 13). A mechanism to explain this could well involve the initial formation of the required quaternary derivative (40) which subsequently loses the benzyl group at N-9. Reactions of this type are known, one example being when 7,9-dimethylhypoxanthinium bromide (42) was heated in dimethylacetamide both 1,7- (43) and 1,9-dibenzylhypoxanthine (44) were among the products



Scheme 13



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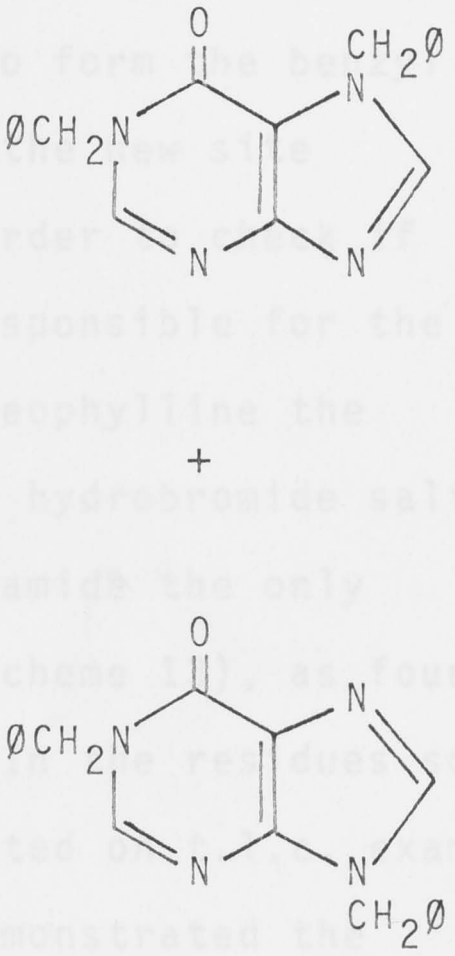


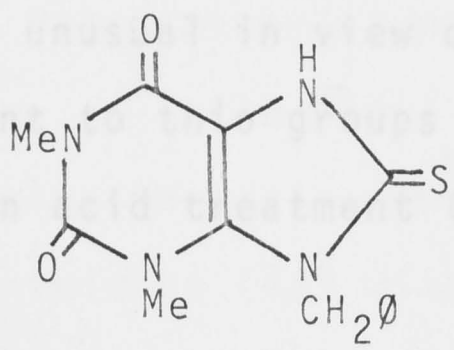
(43)

+

(44)

Scheme 14

isolated (Montgomery, 1966). For this reaction (Scheme 14) it was proposed that the displaced benzyl ion combines with the halogen ion to form the benzyl halide required for benzylation at the new site (Neiman and Bergmann, 1968). In order to check if a similar reaction mechanism was responsible for the interconversion of 9- to 7-benzyltheophylline the 9-benzylpurine was converted to the hydrobromide salt. On heating the salt in dimethylformamide the only product was 7-benzyltheophylline (Scheme 13), as found in the benzyl bromide treatment. In the residues some unchanged 9-benzyl isomer was detected on t.l.c. examination. Previous workers have demonstrated the necessity for the presence of halogen acids in benzyl group migrations of this kind and it was of interest to determine if these reagents were also necessary in this N-9 to N-7 group migration. A repeat heating of 9-benzyltheophylline was therefore carried out in dimethylformamide but in the absence of the hydrogen bromide. In this case only starting material was recovered. It would appear, therefore, that this benzyl group migration falls into the same general category as those previously described by Miyaki (1966) which require the formation and participation of a benzyl halide in the intermolecular rearrangement.



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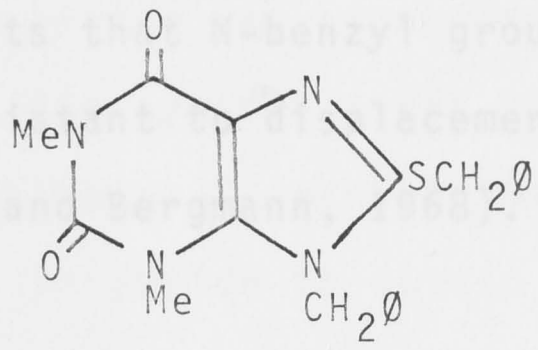


(45)

A somewhat unexpected finding was that when 9-benzyl-8-benzylthiotheophylline (45) was given the same heat treatment in dimethylformamide no migration of the 9-benzyl group was found to occur. Mainly starting material was recovered together with a small amount of the 8-thio analogue (42) which had been formed by S-debenzylation. This observation may not be too unusual in view of reports that N-benzyl groups adjacent to thio groups are resistant to displacement by halogen acid treatment (Neiman and Bergmann, 1968).

F. SYNTHESES AND STUDIES OF 7,8-DIMETHYL-2-METHYLTHIOPURINE

It has been reported (Brown and Ford, 1969) that from the methylation of 2-methylthiopurine with diazomethane, in addition to the 9-methylated purine as the major product, a minor product, the analysis of which corresponded to that of a dimethylated 2-methylthiopurine, was obtained. On the basis of n.m.r., mass and u.v. spectral data this minor product was proposed to be 7,8-dimethyl-2-methylthiopurine, although, at the same time, the possibility that it might be the 1,8-dimethyl-2-methylthio isomer, was not completely ruled out.

In this case diazomethane treatment had not only given the expected N-methylated product but in addition a C-methylated derivative also. Although examples of C-methyl derivatives being formed following

diazomethane treatment have been reported in a number of heterocycles (Review, Gompper, 1963) this appears to be the first example of this kind to be found in the purine series.

With the aim, therefore, of checking whether the structure of the minor product was the one that had been proposed, the unambiguous syntheses of 7,8-dimethyl-2-methylthiopurine was attempted.

The first step in the synthesis was the acylation of 4-amino-5-methylamino-2-methylthiopyrimidine (46) with acetic anhydride to obtain 4-amino-5-(N-methyl)acetamido-2-methylthiopyrimidine (47). Ring-closure of this derivative to the purine (48) required fairly vigorous conditions and was finally achieved by heating in dimethylformamide under reflux conditions.

The physical data of 7,8-dimethyl-2-methylthiopurine (48) were found not to be consistent with those reported for the minor product, the most significant variation being shown between the two melting points, the figures for the minor product (158-159^{o}) being some 40^{o} below those of the synthesised purine (197-198^{o}). A comparison of the ^{1}H n.m.r. data (Table 3) also shows that considerable differences exist in the chemical shifts of the corresponding groups.

Table 3

^{1}H N.m.r. data (δ values) for N-8-dimethyl-2-methylthiopurine and related derivatives ($CDCl_3$/TMS).

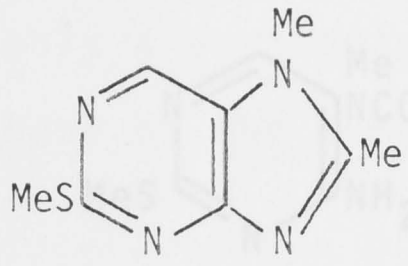
	6H	SMe	8-Me	NMe
N,8-Me_2-2-SMe	8.31	2.84	2.94	4.22
7,8-Me_2-2-SMe	8.61	2.60	2.60	3.77
8,9-Me_2-2-SMe	8.81	2.63	2.61	3.74
1-Me-2-SMe	9.22	2.78	—	4.01

Unfortunately, so far, no derivatives of 3-methyl-2-methylthiopurine are available for ^{1}H n.m.r. spectral examination and therefore the only data available for comparison with that from the N,8-dimethyl-2-methylthiopurine are those from the 1-methyl-, 7-methyl- and 9-methyl-2-methylthiopurines. Only with the ultraviolet spectra is fairly close agreement obtained, the wavelength maxima of the diazomethane methylation product (311 nm and 242 nm) in aqueous solution at neutral pH comparing favourably with those found with 7,8-dimethyl-2-methylthiopurine (309 nm and 238 nm) under similar conditions. This

particular spectral likeness is, however, not greatly significant as the ultraviolet spectra of a number of N- and C-methylated derivatives of methylthiopurines also show absorption maxima around these wavelengths (Brown et al., 1967). As some mass spectral results had also been given (Brown and Ford, 1969) for the minor methylation product a similar examination of the 7,8-dimethyl isomer was made. The fragmentation pattern of the latter (Figure 2) was different from that reported by Brown and Ford (1969). These workers only noted that first HCN and then an acetonitrile fragment was produced from their purine whereas in the case of the synthetic product a methylenethio fragment was initially removed, this then being followed by acetonitrile and subsequently by an HCN fragment.

From the above comparisons it is seen that the minor methylation product is not the same as the 7,8-dimethyl derivative (48). As the 8,9-dimethyl isomer (49) has been already excluded by its synthesis (Brown and Ford, 1969) the remaining possibilities are for either the 1,8-dimethyl (50) or 3,8-dimethyl-2-methylthiopurine (51). Of these two latter derivatives the 3,8-dimethyl might be the more likely as this would be expected to show a lower 6-H chemical shift than shown by 1-methyl-2-methylthiopurine. The high value (9.22 δ) shown by the latter is the highest of the purines studied (Table 3) and in part is due to the

Mass spectrum (70 ev) of



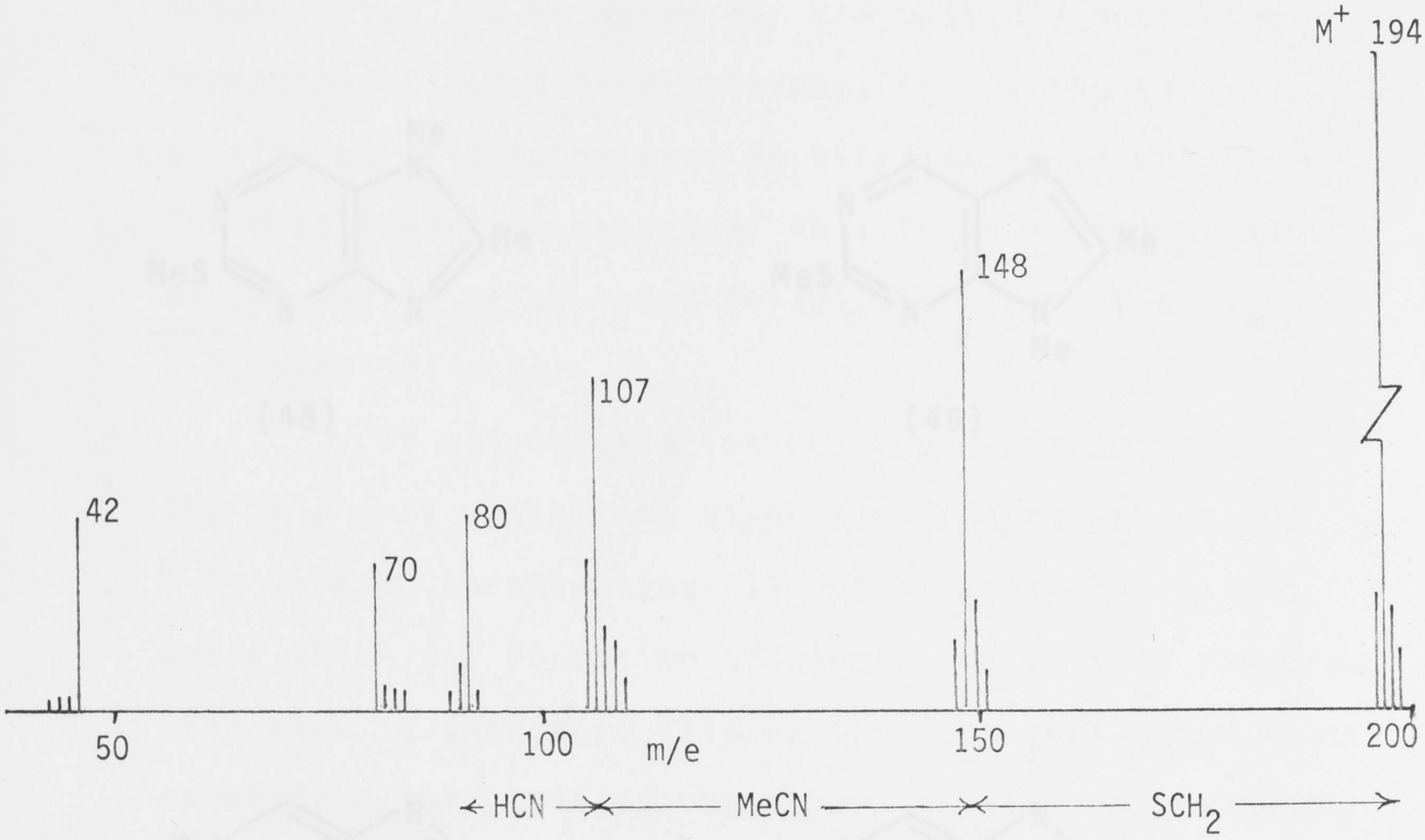
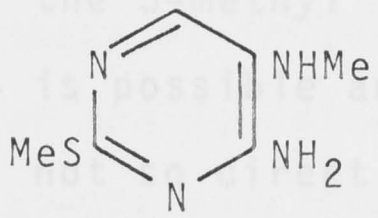


Figure 2



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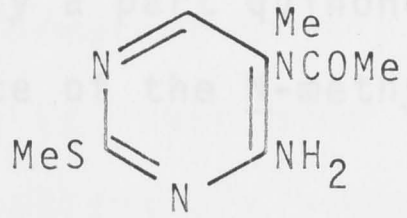

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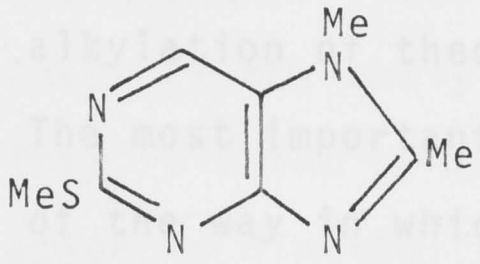

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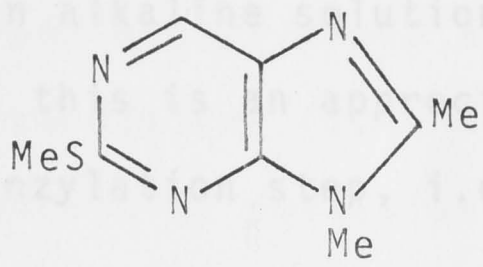

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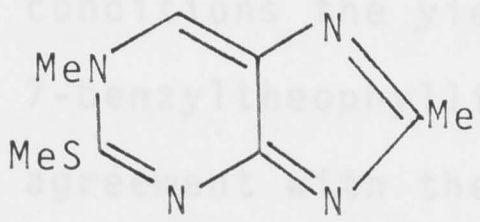

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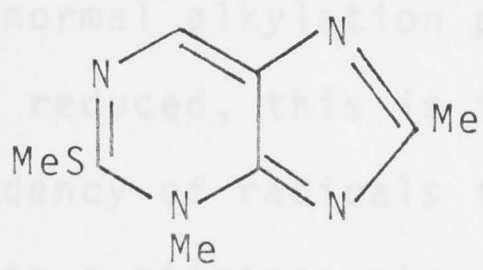


(51)

quinonoid form as in the homologue (50), and the proximity of the N-methyl group. In the case of the 3-methyl isomer (51) only a part quinonoid form is possible and the influence of the N-methyl group not so direct.

G. CONCLUDING REMARKS

A survey of the results obtained from the alkylation and synthetic studies of the benzyltheophylline derivatives allows some conclusions, of a general kind, to be drawn for the possible mode of formation of 7,8-dibenzyltheophylline during the alkylation of theophylline in alkaline solution. The most important aspect of this is an appreciation of the way in which the 8-benzylation step, i.e. the C-alkylation, occurs.

Of all benzylation reactions carried out the only ones which have given significant amounts of 8-benzylated theophyllines are those using conditions under which the formation of benzyl radicals is favoured. This view is supported also by the fact that under these conditions the yield of the normal alkylation product, 7-benzyltheophylline is much reduced, this is in agreement with the known tendency of radicals to prefer attack at a carbon rather than a nitrogen atom. Further indirect support for the radical theory is

given by the results from the quaternisation attempts on 7- and 9-benzyltheophylline. In these no indications of the formation of any of the 7,8-dibenzyl homologue is found even though highly reactive benzyl carbocations are presumed to be generated during the rearrangements studied.

Assuming, therefore, that radical involvement leads to the formation of 8-benzyl-theophylline a possible complicating factor has to be recognised when considering the next step. In the conversion of the 8-benzyl derivative to the 7,8-dibenzyl homologue steric hindrance is found to obstruct the entry of the second benzyl group at N-7. This is shown by the forcing conditions (i.e. alkylation in dimethylformamide containing potassium carbonate under reflux) needed to carry out the reaction. Possibly this factor is also the reason for the failure to isomerise the 8,8-dibenzylpurine to the 7,8-dibenzyl form. As radicals as well as ions are affected by steric considerations this could also explain why ultraviolet irradiation of 7-benzyl-theophylline, in the presence of benzyl bromide, did not give any 7,8-dibenzyltheophylline.

To sum up, from the available evidence it would appear that the most likely pathway to 7,8-dibenzyltheophylline, which arises from benzylation of theophylline, is by an initial attack by radicals occurring at C-8, the benzyl radicals being generated by thermolysis of the benzyl halide. Some of the 8-benzyltheophylline so produced then undergoes an ionic alkylation at N-7 to give the dibenzyl derivative. This latter step seems to be time and temperature dependent, as fairly rigorous conditions were required to overcome the steric barrier of the 8-benzyl group. The alternative mechanism, in which formation of the 8-benzyltheophilline occurs by an N_7(or N_9)-benzyl group migration to C-8, would appear to be ruled out on the practical evidence reported above.

PART II. The 2-Cycloalkylamino-6,9-dimethylpurines

A. INTRODUCTION

Historical

Phleomycin is a copper-containing protein which has antibiotic and antitumour activity (Bradner and Pindell, 1962; Tanaka, et al., 1966; Ishizuka, et al., 1966). This antibiotic, when introduced into cultures of stationary-phase E.coli B cells at doses of <2 µg/ml, induces neither DNA breakdown nor cell death, but on the addition of 8mM caffeine these kinds of changes are initiated. As the same effect can be produced by increasing the phleomycin concentration tenfold the phenomenon is termed "amplification".

In the light of the results derived from the caffeine studies a wide range of purines has been synthesised (Grigg, 1971; Brown, 1972; Grigg, 1972; Badger, 1974; Angyal, 1974) and tested. Although no precise structure-activity relationships emerged from this work amplification effects were generally most pronounced with some 2-alkylthiopurines and corresponding 2-dimethylaminopurines. Furthermore, it was also apparent that an alkyl group at C-6 or C-8 or on an imidazole nitrogen atom, or a combination of these, was beneficial for biological action.

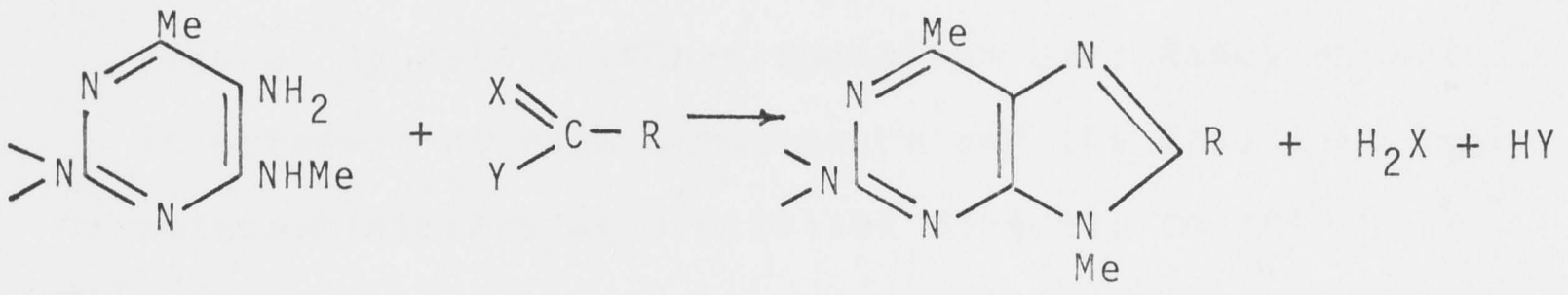
Present Work

In an attempt to improve on the activity of the 2-dialkylaminopurines, some 6,9-dimethylpurines with cycloalkylamino groups at the 2-position were synthesised and tested for their amplifying effect. For the preparation of these purines Traube's synthesis was employed, this involved the introduction of one carbon fragment to bridge the nitrogen atoms of the amino groups at C_4 and C_5 of the pyrimidine ring (Scheme 15). Use of this route enabled the 2-cyclo-alkylamino moiety to be inserted prior to ring closure of the pyrimidine to the purine.

B. SYNTHETIC STUDIES

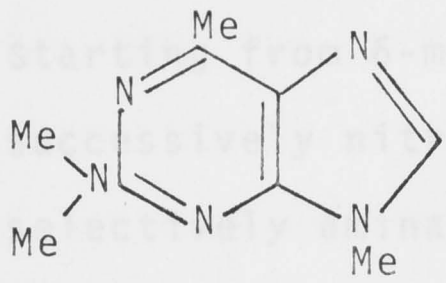
The 2-dimethylamino group in the biologically active 6,9-dimethylpurine (52) was replaced by a series of cyclic analogues, using polymethylene amines with rings containing from four up to seven carbon atoms. In this way, while the basic function of the group at the 2-position was not greatly changed, by the replace-ment of the dimethylamino group, there was, however, a great difference in the steric requirements of the purine. It was felt that the variation of this property with ring size might give some indication of the mode of biological action in the potentiation of phleomycin.

The key intermediate for the preparation of the 2-cycloalkylaminopurines (53, n=2,3,4 and 5)

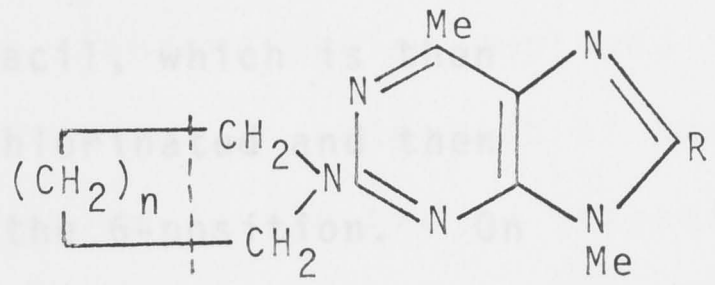


Traube synthesis of purines

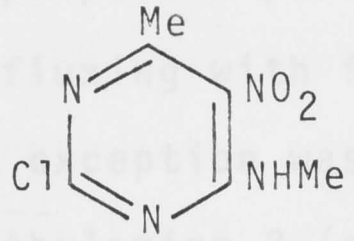
Scheme 15



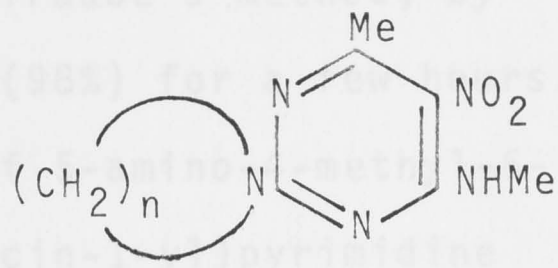
(52)



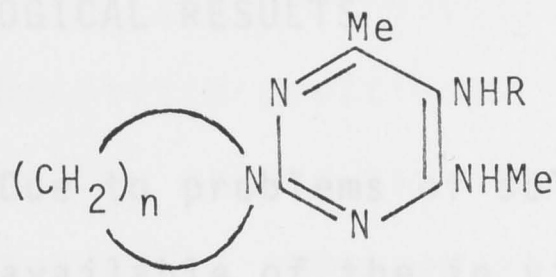
(53)



(54)



(55)



(56)

2-chloro-4-methyl-6-methylamino-5-nitropyrimidine (54) was prepared according to the method in the literature (Brown, England and Lyall, 1966), starting from 6-methyluracil, which is then successively nitrated, chlorinated and then selectively aminated at the 6-position. On treatment of the 2-chloropyrimidine (54) with a cold ethanolic solution of the appropriate cyclic amine the required polymethyleneaminopyrimidines (55, n=4-7) were obtained. These nitropyrimidines were readily reduced by catalytic hydrogenation over Raney nickel in methanol at room temperature and the resulting aminopyrimidines were cyclised directly to the appropriate purines, using Traube's method, by refluxing with formic acid (98%) for a few hours. An exception was the case of 5-amino-4-methyl-6-methylamino-2-(octahydroazocin-1-yl)pyrimidine (56, R=H, n=7) which gave the crude formyl derivative (56, R=CHO, n=7); this was converted to the required purine by heating it under reflux in formamide.

C. BIOLOGICAL RESULTS

Due to problems of solubility complete data are not available of the <u>in vitro</u> testing of the various 2-polymethyleneaminopurines (53) as potentiators of phleomycin. The results obtained so far (Table 4)

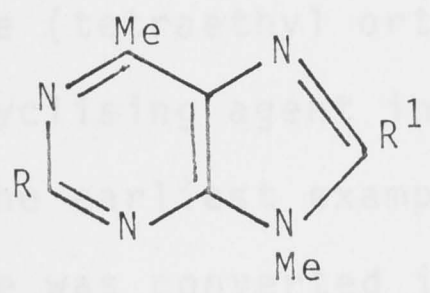
show a reasonably high activity for the 2-piperidinyl derivative (53, R=H, n=3); the activity value of 235 (at 2mM concentration) can be compared with that of 11 (at 2mM) for caffeine the standard purine employed in these tests. It is interesting to note that if the 8-position of the above purine is blocked by insertion of a methyl group, as a means of preventing normal metabolic hydroxylation occurring at this position, then the activity figure for the resulting 6,8,9-trimethyl analogue (53, R=Me, n=3) is reduced to 90 (at 2mM) i.e. to a value 40% of the original figure (Bhushan et al., 1975). The decrease in solubility of the purine, due to the extra methyl group, may, however, be as an important a factor in the lowered biological activity as the effect of blocking the metabolic oxidation step. A noteworthy point in connection with this is that unpublished results from the ongoing programme show that conversion of an 8-methylpurine to the 8-hydroxy-methyl analogue occurs in the metabolic pathway of some of the related 2-alkylthiopurines tested. This suggests that the sought after antimetabolic effect may not be achieved simply by introduction of the methyl group at the unsubstituted position. Although the piperidino and morpholino groups have approximately the same size and configuration replacement of the former by the other leads to a significant reduction in activity (40/2mM) in both di- and tri-methylpurines.

When the 2-piperidinopurines are compared with the 2-dimethylamino analogues (52, R=H and Me) activities of the same magnitude are found but the order for the dimethyl and trimethyl derivatives is completely reversed.

These results demonstrate, therefore, the difficulty in proposing a rationale for the potentiating activity of the 2-alkylamino derivatives. The presence of a similarly shaped group is shown, by the significantly different results from the piperidino and morpholino analogues, not to be essential. Likewise, the contrast between the piperidino and dimethylamino values indicates that even though the two parent amines have similar pK_a's (piperidine 11.2, dimethylamine 10.9) basicity alone is not a major factor in the activity criteria.

Table 4

Potentiating effect of 2-Alkylamino-6,9-dimethyl- and 6,8,9-trimethylpurines on the activity of Phleomycin *in vitro* towards *E.coli*



R	R^1	Activity, concentration*
piperidino [a]	H	233, 2mM
piperidino [a]	Me	90, 2mM
dimethylamino [b]	H	85, 8mM
dimethylamino [b]	Me	225, 8mM
morpholino	H	40, 2mM
morpholino [a]	Me	40, 2mM

(Note*. Caffeine, as standard, activity 30, 8mM and 11, 2mM)

[a] Bhushan (1975).

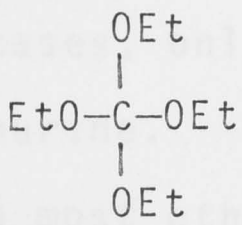
[b] Brown (1972).

PART III The 8-Alkoxy-6-aminopurines

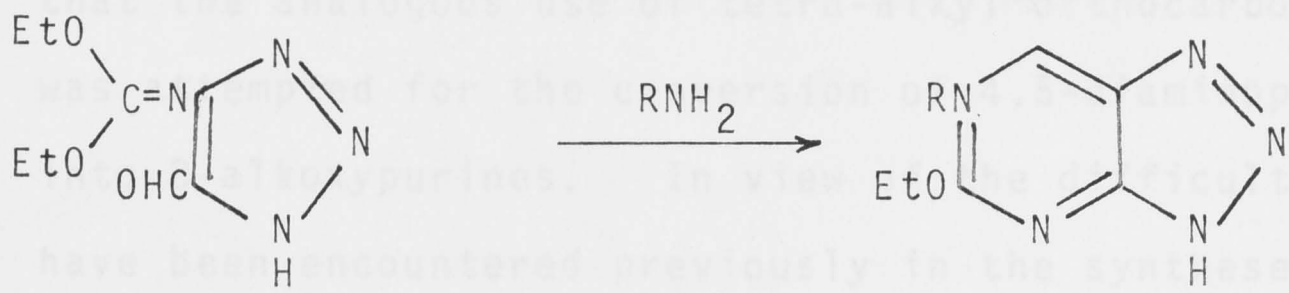
A. INTRODUCTION

It was only some two years ago that the use of tetraethoxymethane (tetraethyl orthocarbonate) (57) was pioneered as a cyclising agent in the synthesis of heterocycles. In the earliest examples 5-amino-4-formyl-1,2,3-triazole was converted into the corresponding 5-diethoxymethyleneamino derivative (58) this upon subsequent treatment with amines then undergoing cyclisation to give 5-ethoxy-v-triazolo-[4,5-d]pyrimidines (59) (Albert and Taguchi, 1973). In other studies 5-amino-4-hydrazinopyrimidines were similarly converted initially into their 4-diethoxymethylenehydrazino analogues (60) prior to their cyclisation and subsequent oxidation to 3-ethoxypyrimido[5,4-e]-as-triazines (61) (Brown and Lynn, 1973).

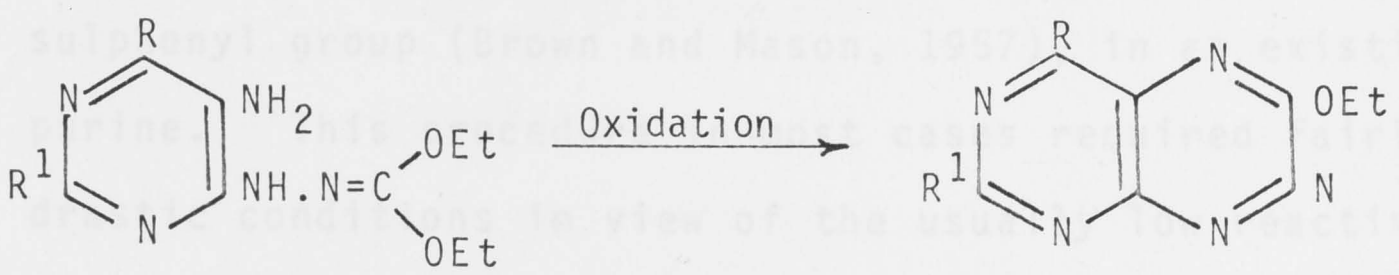
Orthoesters, which are the trialkoxymethane analogues of the orthocarbonates, have been extensively used to cyclise 4,5-diaminopyrimidines to the corresponding 8-unsubstituted or 8-alkylpurines depending upon the orthoester employed. For example, purines having a methyl or ethyl substituent at C-8 have been prepared from triethyl orthoacetate and triethyl orthopropionate, respectively (Koppel and Robins, 1958; Prasad, Noell, and Robins, 1959: Montgomery and Temple, 1960; Pfleiderer, 1961).



(57)



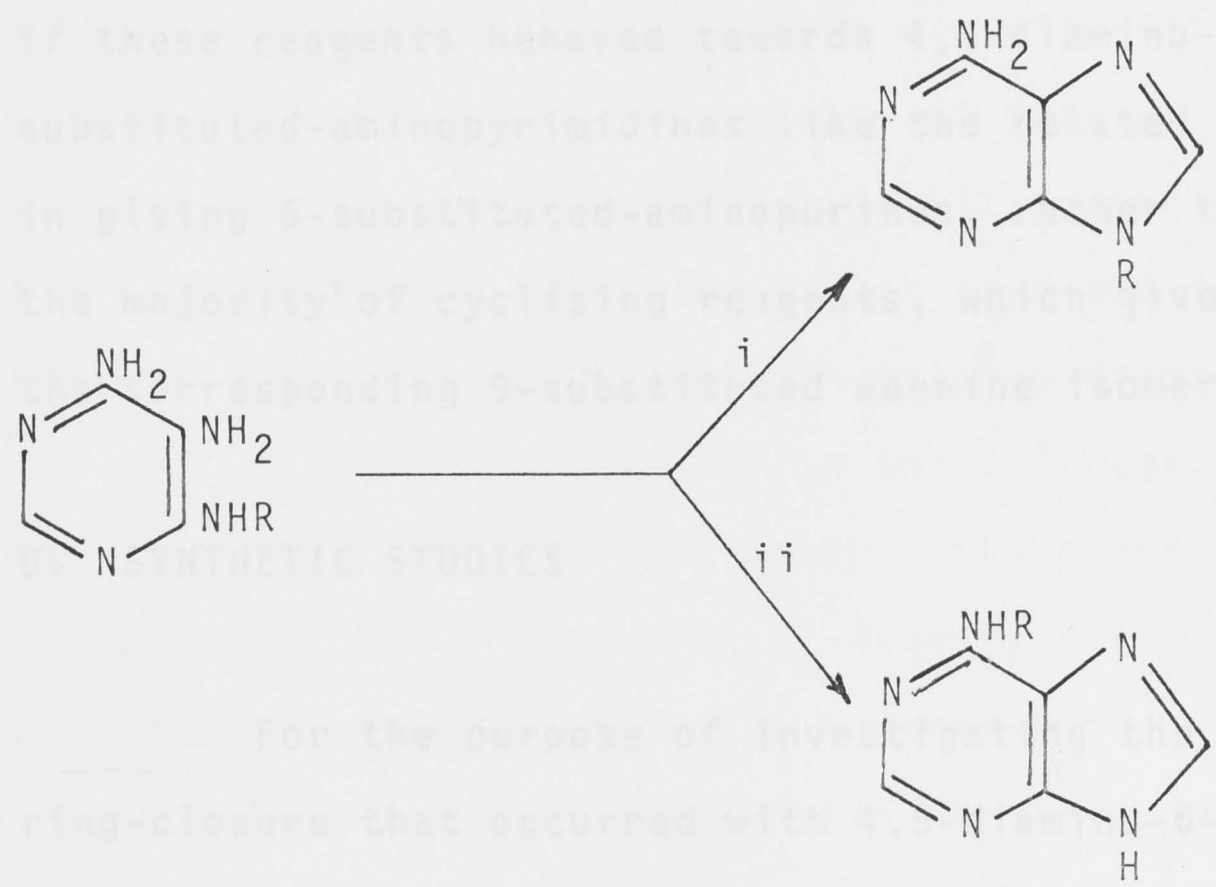
(58) (59)



(60) (61)

A notable feature of orthoester cyclisations in purine synthesis is that when employed with 4,5-diamino-6-monosubstituted-aminopyrimidines, the major, and in most cases, only product is the 6-substituted-aminopurine. This is contrary to the situation found with most other cyclising agents which give the isomeric 9-substituted adenine as the main product of the ring-closure (Scheme 16).

It was only recently (Brown and Lynn, 1974) that the analogous use of tetra-alkyl orthocarbonates was attempted for the conversion of 4,5-diaminopyrimidines into 8-alkoxypurines. In view of the difficulties which have been encountered previously in the syntheses of 8-alkoxypurines, the advantage of this reagent for allowing the direct introduction of an alkoxy group at the 8-position is apparent. Prior to this no generally applicable or convenient methods existed, the most usual route to 8-alkoxy derivatives was by the displacement by an alkoxy group of a suitable 8-substituent, this being either a halogen (Robins, 1958), methylthio or methylsulphonyl group (Brown and Mason, 1957), in an existing purine. This procedure in most cases required fairly drastic conditions in view of the usually low reactivity of a group at the 8-position towards nucleophilic displacement. Of even less general applicability was the method of O-alkylation of 8-oxopurines, this procedure does not appear to be effective except with certain derivatives of uric acid.



i. Usual cyclising agents

ii. Orthoesters

Scheme 16

Present Work

Arising from the initial encouraging results which had been obtained with this class of reagent in 8-alkoxypurines syntheses by Brown and Lynn (1974) further studies have been undertaken to explore further the versatility of orthocarbonates for this purpose. One aspect given particular attention was to ascertain if these reagents behaved towards 4,5-diamino-6-substituted-aminopyrimidines like the related orthoesters, in giving 6-substituted-aminopurines, rather than as with the majority of cyclising reagents, which give rise to the corresponding 9-substituted adenine isomers.

B. SYNTHETIC STUDIES

For the purpose of investigating the type of ring-closure that occurred with 4,5-diamino-6-substituted-aminopyrimidines, i.e. whether ring-closure took place between the 4- and 5-primary amino groups or between the 5-amino and 6-substituted (secondary) amino groups; three triaminopyrimidines were prepared each one having a different size secondary amino group. These were the 4,5-diamino-6-benzylaminopyrimidine (62, R=$CH_2Ø$) together with the 6-methylamino (62, R=Me) and 6-anilino (62, R=Ø) analogues.

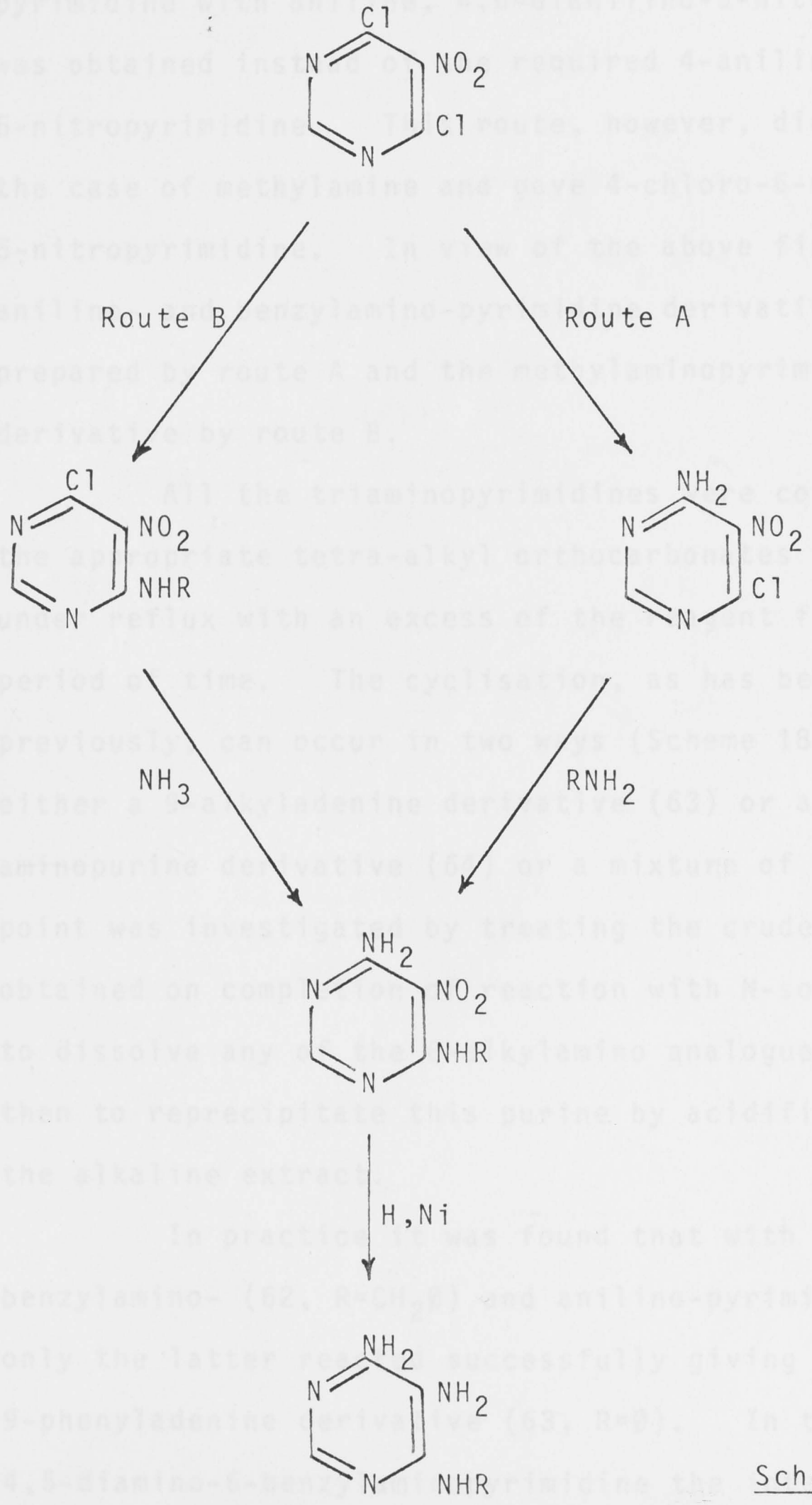
The key intermediate for the preparation of these triaminopyrimidines, 4,6-dichloro-5-nitropyrimidine, was obtained using the method reported in the literature (Boon, Jones and Ramage, 1951).

Two possible routes were considered for the preparation of the required triaminopyrimidines (Scheme 17); in the first route one of the halogen atoms is initially replaced by an amino group, using ammonia solution (route A), whereas in the other approach the secondary amino group is inserted first (route B).

The intermediate 6-amino-4-chloro-5-nitropyrimidine (route A) was found to be quite difficult to obtain in good yield by the method of Boon et al. (1951) because of the formation of substantial amounts of 4,6-diamino-5-nitropyrimidine which occurred through concomittant ammonolysis of both halogen atoms.

It had been reported in the literature (Segal and Shapiro, 1959) that the chance of bis-ammonolysis occurring in the preparation of 4-amino-6-chloro-5-nitropyrimidine was reduced to a minimum if the reaction was carried out in tetrahydrofuran in the presence of sodium bicarbonate. This procedure when used gave the required monoamino compound which was then treated with the required alkylamine, following the published procedure (Daly and Christensen, 1956), to give the desired 4-alkylamino-6-amino-5-nitropyrimidines. These 5-nitro derivatives were then easily reduced by hydrogenation over Raney nickel to the corresponding 4-alkylamino-5,6-diaminopyrimidines.

The alternative route (B) differs from the previous one in that the 4,6-dichloropyrimidine is treated first with the required alkylamine and the


Cl
N
NO2
Cl
N
Route B
Route A
Cl
N
NO2
NHR
N
NH2
N
NO2
Cl
N
NH3
RNH2
NH2
N
NO2
NHR
N
H,Ni
NH2
N
NH2
NHR
N

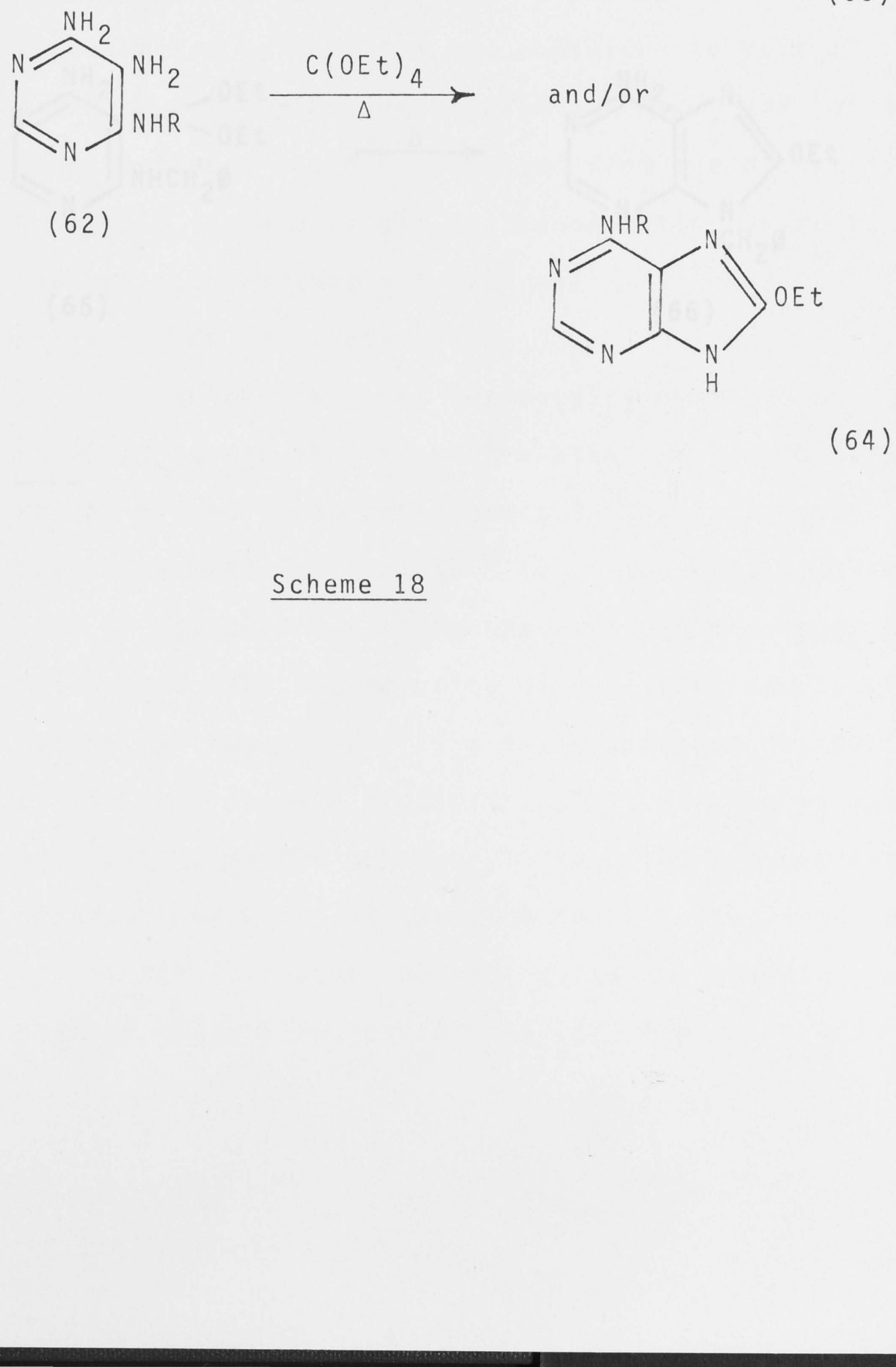
(62)

Scheme 17

halogen then is replaced with ammonia. However, when an attempt was made to treat 4,6-dichloro-5-nitro-pyrimidine with aniline, 4,6-dianilino-5-nitropyrimidine was obtained instead of the required 4-anilino-6-chloro-5-nitropyrimidine. This route, however, did work in the case of methylamine and gave 4-chloro-6-methylamino-5-nitropyrimidine. In view of the above findings the anilino- and benzylamino-pyrimidine derivatives were prepared by route A and the methylaminopyrimidine derivative by route B.

All the triaminopyrimidines were condensed with the appropriate tetra-alkyl orthocarbonates by heating under reflux with an excess of the reagent for a prolonged period of time. The cyclisation, as has been stated previously, can occur in two ways (Scheme 18) to give either a 9-alkyladenine derivative (63) or a 6-alkyl-aminopurine derivative (64) or a mixture of both. This point was investigated by treating the crude product obtained on completion of reaction with N-sodium hydroxide, to dissolve any of the 6-alkylamino analogue formed, and then to reprecipitate this purine by acidification of the alkaline extract.

In practice it was found that with the benzylamino- (62, R=$CH_2Ø$) and anilino-pyrimidines (62, R=Ø) only the latter reacted successfully giving the 9-phenyladenine derivative (63, R=Ø). In the case of 4,5-diamino-6-benzylaminopyrimidine the intermediate 5-diethoxymethyleneamino derivative (65) was isolated. Attempts to cyclise this by fusion in a metal bath did

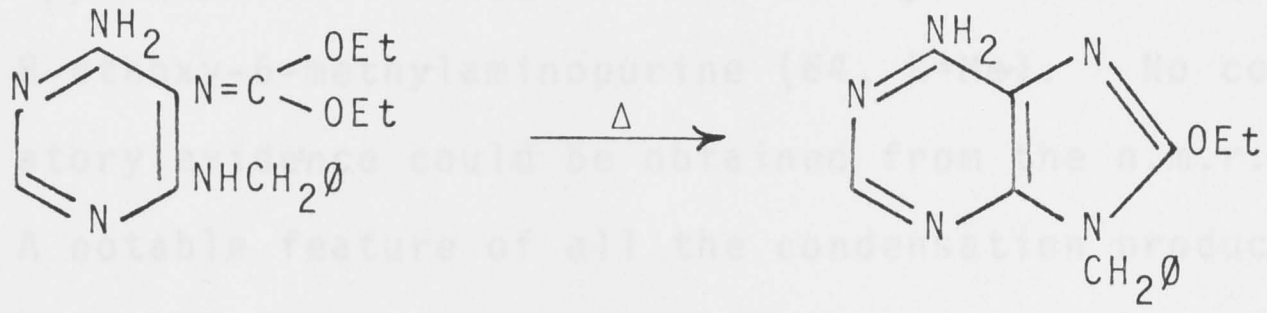


(62)

(63)

(64)

Scheme 18



(65) (66)

not give 9-benzyl-8-ethoxyadenine (66), only starting material being recovered. With the methylamino-pyrimidine (62, R=Me) two products, separable by alkali treatment, were obtained. Analytical data showed the alkali insoluble derivative to be the 8-ethoxy-9-methyladenine (63, R=Me), this being confirmed by ^{1}H n.m.r. data. The analysis of the material recovered on acidification of the alkaline solution approximated to that for the monohydrated form of 8-ethoxy-6-methylaminopurine (64, R=Me). No confirmatory evidence could be obtained from the n.m.r. spectrum. A notable feature of all the condensation products was that no satisfactory nitrogen analysis could be obtained for any derivative.

On the basis of the results obtained so far, it would appear that the tetra-alkyl orthocarbonates do not resemble the orthoesters in their condensation reactions with 4,5-diamino-6-substituted-aminopyrimidines. They do not give 6-alkylaminopurines as the major product but behave like the majority of cyclising agents used in purine syntheses and afford 9-alkyladenine derivatives as the sole or main products. This finding is quite interesting as the tendency to form the 6-substituted-aminopurines would be expected to be quite large in view of the steric factors operating, as for example, in the case of the anilino-pyrimidine (62, R=Ø).

Attempts to ring-close the above triaminopyrimidines using either the tetramethoxy or tetrapropoxy orthocarbonates were unsuccessful. No identifiable products could be isolated from these reactions.

PART IV. Experimental

Microanalyses were carried out by the Australian National University Analytical Services Unit. Solids for analysis were dried at 100° unless otherwise stated. Melting points were taken in Pyrex capillaries and are corrected.

All compounds were examined for the presence of impurities or isomers by thin layer chromatography (silica or alumina plates). Where possible, solids were recrystallised to constant melting points.

^{1}H N.m.r. spectra were measured at 60 MHz and 35° with a Varian T-60A spectrometer and were determined in $(CD_3)_2SO$ or $CDCl_3$, with tetramethylsilane as internal standard. Mass spectra were measured on an A.E.I. MS 9 instrument.

Infrared spectra were measured as Nujol mulls on a Unicam SP 200 or SP 1000 instrument. Ultraviolet spectra were recorded with a Unicam SP 800 or SP 1800 instrument. The ultraviolet source in the irradiation experiments was a 128W Oliphant chamber reactor equipped with interchangeable short (254 nm) or long (365 nm) wavelength lamps.

All new compounds are underlined at their first mention in the body of the text of this Experimental Section.

A. BENZYLTHEOPHYLLINES AND INTERMEDIATES

Benzylation of Theophylline.—To a solution of theophylline (5 g) and sodium hydrogen carbonate (2.3 g) in water (100 ml) was added benzyl bromide (4.7 g) and the mixture heated under reflux for 4 h. The crystalline product, which separated on cooling, was stirred with N sodium hydroxide solution, filtered and recrystallised from ethanol giving 7-benzyltheophylline, m.p. 156-157° (Traube, 1923: 158°). Acidification of the alkaline filtrate (pH 5) with acetic acid gave a precipitate of 8-benzyltheophylline (0.07 g) m.p.289-290° (Hager et al., 1954: 289-290°). The ethanolic mother liquors from recrystallisation of the 7-benzyl derivative were evaporated and the residue taken up in chloroform and applied to a preparative t.l.c. plate (Merck type 60 silica) and run in ethyl acetate. Extraction of the lower of the two main bands with hot chloroform gave more of the 7-benzyl isomer (total yield 2.30 g) while from the upper band 7,8-dibenzyltheophylline (0.04 g), m.p. 120-121° was recovered (Found: C, 70.3; H, 5.7; N, 15.9. $C_{21}H_{20}N_4O_2$ requires C, 70.0; H, 5.5; N, 15.5%). Evaporation of the aqueous filtrate from the original reaction mixture gave only unreacted theophylline (0.67 g). Virtually identical yields of the 7-, 8- and 7,8-benzylated derivatives, to those given above, were formed when the sodium salt of theophylline and benzyl bromide were reacted in water at 100°. In addition to m.p.s further

confirmation of 7- and 8-benzyltheophylline was obtained from their spectral comparisons (i.r., ^{1}H n.m.r.) with those of authentic specimens.

8-Benzyltheophylline.—The solid, obtained on heating a mixture of 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione (5 g, 1 mole) and phenylacetic acid (5 g, 1.25 mole) at 170-180°, was cyclised in sodium hydroxide solution (9%), as described by Hager et al. (1954), to the required 8-benzyltheophylline (3.3 g) m.p.290-292° (Hager et al., 1954: 289°).

Benzylation of Theophylline in the presence of Ultraviolet light.—To a solution of theophylline (5 g) and sodium hydrogen carbonate (2.3 g) in water (100 ml) was added benzyl bromide (4.7 g) and the mixture was heated for 12 h in the presence of ultraviolet light by means of superheated steam passing through the coils dipped in the reaction mixture; the maximum temperature of the solution attained was 92°. The solid which separated out on cooling was filtered off and treated with cold chloroform and the chloroform extracts concentrated after drying over anhydrous sodium sulphate. The 8-benzyltheophylline which separated out was removed and the chloroform mother liquors run on a silica t.l.c. plate. When the topmost band was extracted with chloroform 7,8-dibenzyltheophylline (1.50 mg) m.p. 120-121° was obtained on evaporation of the extracts.

The aqueous filtrate on standing threw out a solid which was found to be a mixture of 7-benzyl- and 8-benzyltheophylline. The former purine remained on treatment of the mixture with alkali from which 8-benzyltheophylline was recovered on acidification of the solution. Further working up of the aqueous filtrate gave only theophylline. Respective yields were 7-benzyltheophylline (0.40 g), 8-benzyltheophylline (0.14 g) and theophylline (2.30 g).

When the experiment was repeated at 74° for 12 h, no 7,8-dibenzyltheophylline could be detected, 7-benzyltheophylline (0.6 g), 8-benzyltheophylline (0.5 g) and unreacted theophylline (2.55 g) only were recovered.

Action of Crotyl bromide on Theophylline.—Sodium theophyllinate (1 g) in water (50 ml) was stirred with crotyl bromide (0.66 g) for 6 h. Crystallisation of the precipitate from methanol gave 8-crotyltheophylline (0.5 g) m.p. 232-234°; n.m.r. δ(DMSO) 1.60 (m, C-Me), 3.20, 3.38 (s, N-Me), 3.38 (s, CH_2), 5.56 (m, CH=CH). Evaporation of the aqueous filtrate from the reaction mixture gave only unreacted theophylline (0.12 g) while the methanolic mother liquors from the crystallisation were found to contain only minor amounts of 8-crotyltheophylline. All the precipitated 8-crotylpurine was alkali soluble, i.e. no 7-alkylated isomer was present. This was confirmed by t.l.c. examination of the products.

Benzylation of 8-Benzyltheophylline.—The sodium salt of 8-benzyltheophylline, prepared by evaporating to dryness a solution of the purine (1 g) in N sodium hydroxide (9 ml) and water (25 ml), was heated (oil bath) with benzyl chloride (0.8 ml) in dimethylformamide (25 ml) under reflux for 4 h. After removal of the solvent the residue was crystallised from ethanol to give 7,8-dibenzyltheophylline (0.7 g), identical (m.p., i.r., n.m.r.) with that previously described.

When the benzylation experiment in aqueous solution was attempted in the absence of sodium hydroxide, only starting material was recovered.

6-Amino-5-benzylamino-1,3-dimethylpyrimidine-2,4-dione.—5,6-Diamino-1,3-dimethylpyrimidine-2,4-dione (5 g) and benzaldehyde (3.5 g) were stirred in ethanol (100 ml) at room temperature for 2 h, using the procedure of Traube and Nithack (1906). The 6-amino-5-benzylideneamino-1,3-dimethylpyrimidine-2,4-dione (4.5 g) which separated out had a m.p. 218-219°.

A solution of 6-amino-5-benzylideneamino-1,3-dimethylpyrimidine-2,4-dione (5 g) in ethanol (150 ml) was hydrogenated over Raney nickel until hydrogen uptake was completed. After removal of the catalyst and evaporation of the solvent the residue was crystallised from ethanol giving 6-amino-5-benzylamino-1,3-dimethylpyrimidine-2,4-dione (3.5 g) m.p. 184-186° (Found: C, 60.2; H, 5.8; N, 21.8. $C_{13}H_{16}N_4O_2$ requires C, 60.0; H, 6.2; N, 21.5%).

Following Sugimoto and Matsuura's method (1968) a mixture of 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione (5 g) and benzaldehyde (3.4 g) in ethanol (150 ml) when hydrogenated under the foregoing conditions also gave the same 6-amino-5-benzylaminopyrimidine (m.p., i.r., n.m.r.).

Conversion of 6-Amino-5-benzylamino-1,3-dimethylpyrimidine-2,4-dione into 7-Benzyltheophylline.—After heating a solution of the above 5-benzylamino derivative (0.2 g) in formic acid (98%, 15 ml) under reflux for 4 h and then evaporating to dryness only the 5-(N-benzyl)formamidopyrimidine was obtained. This crude product was taken up in formamide (5 ml) containing hydrochloric acid (0.1 ml) and heated at 170° (oil bath) for 1 h then taken down in vacuo to dryness. On crystallisation of the residue from ethanol 7-benzyltheophylline (0.1 g) was obtained, identical (m.p., i.r.) with that derived from benzylation of theophylline, described above.

Attempted preparation of 7,8-Dibenzyltheophylline.—(a) With phenylacetic acid: 6-Amino-5-benzylamino-1,3-dimethylpyrimidine-2,4-dione (0.1 g) and phenylacetic acid (0.06 g) were fused at a temperature of 200° on a metal bath for 2 h. After cooling, the crude product was crystallised from ethanol and identified by i.r., n.m.r. and m.p. (288°) as 8-benzyltheophylline.

(b) With phenylacetamide: 6-Amino-5-benzylamino-1,3-dimethylpyrimidine-2,4-dione (0.1 g) and phenylacetamide (0.07 g) were fused at 250-260^{0} for 3 h, cooled and the solid melt recrystallised from ethanol. The i.r. and m.p. (287-289^{0}) confirmed it to be 8-benzyltheophylline.

(c) With benzyl cyanide: When 6-amino-5-benzylamino-1,3-dimethylpyrimidine-2,4-dione (0.1 g) and benzyl cyanide (10 ml) were heated at 270^{0} for 4 h, then cooled, a yellow solid emerged, identified by i.r. and n.m.r. as 8-phenyltheophylline.

6-Amino-5-benzylaminopyrimidine-2,4-dione.— Following Traube's (1923) method 5,6-diaminopyrimidine-2,4-dione (5 g) was dissolved in hot water (250 ml) and benzaldehyde (2 g) then added with stirring. When the addition was completed the solution was brought to pH 7 with 1N sodium hydroxide and stirred for 4-5 h. The precipitated 6-amino-5-benzylideneaminopyrimidine-2,4-dione (3.5 g) was purified by reprecipitation from alkali with acetic acid, m.p. 330^{0} (dec.).

6-Amino-5-benzylideneaminopyrimidine-2,4-dione (2 g) was suspended in water (70 ml) and 5% sodium amalgam (60 g) was added (Traube, 1923). The contents of the flask were shaken for 5 h and the solution decanted from the mercury residue. On neutralisation of the aqueous layer with acetic acid 6-amino-5-benzylamino-pyrimidine-2,4-dione (1.1 g) precipitated out.

Further purification by reprecipitating from alkaline solution gave a product with m.p. 264-266° (Found: C, 56.6; H, 5.2; N, 24.4. Calculated for $C_{11}H_{12}N_4O_2$: C, 56.9; H, 5.2; N, 24.1%).

6-Amino-5-(N-benzyl)phenylacetamidouracil.—Under fusion conditions (230°, 4 h) 6-amino-5-benzylaminopyrimidine-2,4-dione (1 g) and phenylacetic acid (0.72 g) gave the 5-(N-benzyl)phenylacetamido derivative (0.6 g), m.p. >300°, obtained pure by reprecipitation from alkali with acetic acid (Found: C, 65.1; H, 5.0; N, 15.5. $C_{19}H_{18}N_4O_3$ requires: C, 65.1; H, 5.1; N, 16.0%).

7,8-Dibenzylxanthine.—Heating a solution of the foregoing 5-(N-benzyl)phenylacetamido derivative (1 g) in 2N sodium hydroxide for 8 h, followed by neutralization with acetic acid (pH 6) gave 7,8-dibenzylxanthine (0.5 g) m.p. 270° (dec.)(Found: C, 66.9; H, 4.9; N, 16.2. $C_{19}H_{16}N_4O_2.0.5H_2O$ requires: C, 66.9; H, 5.0; N, 16.4%).

Attempted methylation of 7,8-dibenzylxanthine.—When the methylation of 7,8-dibenzylxanthine in sodium hydroxide with methyl iodide was attempted at room temperature only the starting material was recovered. Complete solution of the xanthine derivative in the alkaline medium could not be obtained.

Attempted Condensation of 5,6-Diamino-1,3-dimethylpyrimidine-2,4-dione with the bisulphite adduct of Dibenzyl Ketone.—A saturated solution of sodium bisulphite in water and dibenzyl ketone in ethanol were mixed and shaken for a short time, the crystalline bisulphite adduct of dibenzyl ketone separating out.

5,6-Diamino-1,3-dimethylpyrimidine-2,4-dione (0.1 g) and the bisulphite adduct (0.23 g) were refluxed in ethanol (15 ml) for 2 h, then filtered. The 8-benzyl-theophylline, which separated out on chilling the filtrate, had a m.p. 288-290^{o} and gave an infrared spectrum superimposable with that of the authentic specimen.

This condensation, when attempted in n-butanol and methanol, also gave 8-benzyltheophylline as product.

Attempted Condensation of 5,6-Diamino-1,3-dimethylpyrimidine-2,4-dione with bisulphite adduct of Benzylmethyl ketone.—The bisulphite adduct of benzyl-methyl ketone was prepared in a similar manner to that described for dibenzyl ketone: 5,6-diamino-1,3-dimethyl-pyrimidine-2,4-dione (0.23 mole) and the bisulphite adduct of benzylmethyl ketone (0.28 mole) were refluxed in n-butanol for 6 h. After removal of the solvent the residue was recrystallised from ethanol giving 8-methyl-theophylline, having the same m.p., i.r. and n.m.r. as an authentic specimen.

Synthesis of 8-Methyltheophylline.—5,6-Diamino-1,3-dimethylpyrimidine-2,4-dione was heated under reflux with acetic anhydride containing a catalytic amount of pyridine (0.1 ml), as described by Bredereck et al. (1953), giving 8-methyltheophylline, m.p. 330-332°.

Condensation of 6-Amino-1,3-dimethyl-5-nitroso-pyrimidine-2,4-dione with bisulphite adduct of dibenzyl ketone.—6-Amino-1,3-dimethyl-5-nitrosopyrimidine-2,4-dione (1 mole) and the bisulphite adduct of dibenzyl ketone (1.1 mole) were heated under reflux in dimethyl-formamide for 2 h. At the end of this period the contents of the flask were evaporated to dryness and the residue recrystallised from ethanol giving 8-benzyltheophylline with the same m.p., i.r. as an authentic sample.

4-Hydroxy-1,3-dimethyluracil.—This was prepared by following the method of Pfleiderer et al. (1958). N,N'-Dimethylurea (88 g), malonic acid (120 g) and acetic acid (190 ml) were heated with stirring at 60-70° till solution was obtained. Acetic anhydride (400 ml) was then added and the temperature raised slowly to 90° and kept there for 6 h. After leaving overnight the mixture was evaporated to a syrup and to this warm ethanol (100 ml) was added. The resulting crystalline product was filtered off, washed with ethanol, and

heated with hydrochloric acid (120 ml) and water (150 ml) under reflux for 2 h. On cooling long needles of the 4-hydroxyuracil, m.p. 120-121° (Pfleiderer, 1958: 123-124°) separated out.

6-Chloro-1,3-dimethyluracil.—4-Hydroxy-1,3-dimethyluracil(50 g) was treated with water (16 ml) and then phosphorus oxychloride was added slowly (400 ml). When the addition was complete the mixture was heated under reflux (40 min) on an oil bath. After removal of the excess of phosphoryl chloride the syrup was poured onto ice with stirring. The resulting solution was filtered and the filtrate extracted with chloroform (5 x 100 ml), the combined extracts dried over sodium sulphate and evaporated to dryness. The required chlorouracil, m.p. 110-111° was obtained on crystallisation of the residue from water.

2-Amino-1,3-diphenylpropane.—Dibenzyl ketone (5 g) was mixed with an equivalent of hydroxylamine hydrochloride in ethanol (15 ml) containing a drop of hydrochloric acid. The ketoxime was obtained as a crystalline solid, m.p. 120-121° (Francis, 1899: 119-125°) on heating the mixture under reflux (1.5 h) and then cooling.

The ketoxime was reduced by hydrogenation over Raney nickel in ethanol to give 2-amino-1,3-diphenylpropane. This was purified by conversion to the hydrochloride, m.p. 205°, yield 2.8 g.

6-(1-Benzyl-2-phenyl)ethylamino-1,3-dimethyl-pyrimidine-2,4-dione.—2-Amino-1,3-diphenylpropane (2 g, 1 mole) was mixed with 4-chloro-1,3-dimethyl-uracil (1.7 g) and heated in dimethylformamide (15 ml) for 1.5 h. On chilling the reaction mixture 6-(1-benzyl-2-phenyl)ethylamino-1,3-dimethylpyrimidine-2,4-dione separated out, m.p. 272-273°, yield 0.8 g (Found: C, 72.3; H, 6.4; N, 12.2. $C_{21}H_{23}N_3O_2$ requires: C, 72.2; H, 6.6; N, 12.0%).

Attempted C-nitrosation of 6-(1-benzyl-2-phenyl) ethylamino-1,3-dimethylpyrimidine-2,4-dione.—Following the method of Goldner et al. (1966) 6-(1-benzyl-2-phenyl)ethylamino-1,3-dimethylpyrimidine-2,4-dione (0.05 g) was treated with isoamyl nitrite (0.15 ml) and ethanolic hydrogen chloride and left overnight whereupon 6-(1-benzyl-N-nitroso-2-phenyl)ethylamino-1,3-dimethyl-pyrimidine-2,4-dione was obtained, m.p. 121-122° (Found: C, 66.2; H, 6.2; N, 14.5. $C_{21}H_{22}N_4O_3$ requires: C, 66.7; H, 5.8; N, 14.8%). The i.r. spectrum showed the absence of an —NH— band and the lack of colour in the product was further confirmation that N- rather than C-nitrosation had occurred.

6-Chloro-1,3-dimethyl-5-nitropyrimidine-2,4-dione.—The nitration of 6-chloro-1,3-dimethyluracil was carried out as described by Liao and Chang (1964). 6-Chloro-1,3-dimethyluracil was added slowly to sulphuric acid cooled in ice water. Fuming nitric acid (d, 1.50) was

then added slowly at such a rate as to hold the temperature below 10°. At the end of the reaction the mixture was poured onto ice and stirred vigorously. The aqueous layer was extracted with chloroform, the extracts dried over sodium sulphate and then evaporated to dryness to give 6-chloro-1,3-dimethyl-5-nitropyrimidine-2,4-dione.

6-Benzylamino-1,3-dimethyl-5-nitropyrimidine-2,4-dione.—Benzylamine (12.4 g) was added to a well stirred solution of 6-chloro-1,3-dimethylpyrimidine-2,4-dione (13 g) in ethanol (150 ml). After 3 h the precipitate was filtered off and suspended in water (100 ml) and the mixture extracted with chloroform (3 x 25 ml) and the extracts dried (sodium sulphate). The solid remaining on evaporation of the chloroform was crystallised from ethanol giving the 6-benzylamino-5-nitropyrimidine (11 g), m.p. 140-141° (Found: C, 53.5; H, 4.8; N, 19.2. $C_{13}H_{14}N_4O_4$ requires: C, 53.8; H, 4.8; N, 19.3%).

Reduction of 6-benzylamino-1,3-dimethyl-5-nitropyrimidine-2,4-dione.—A solution of 6-benzylamino-1,3-dimethyl-5-nitropyrimidine-2,4-dione (5 g) in ethanol (250 ml) was hydrogenated to completion over Raney nickel catalyst. The product (2.4 g) remaining after removal of catalyst and solvent evaporation was 8-phenyltheophylline (i.r., n.m.r.). The same purine was identified as product when either acid stannous chloride or alkaline ferrous sulphate were used as reducing agents for the nitropyrimidine.

8-Phenyltheophylline.—The melt obtained by fusing 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione and benzoic acid at 180-200° was cyclised in 9% sodium hydroxide solution, as described by Hager et al. (1954), to give 8-phenyltheophylline, m.p. >350°.

Attempted Transamination of 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione.—The melt, resulting from heating a mixture of the 4,5-diaminopyrimidine (0.2 g), benzylamine hydrochloride (0.16 g) and benzylamine (10 ml) for 4 h at 150° (metal bath), when cooled and triturated with water gave 8-phenyltheophylline (0.12 g), m.p. >350°, in crystalline form from acetic acid (Found: C, 60.8; H, 4.8; N, 21.6. Calculated for $C_{13}H_{12}N_4O_2$: C, 60.9; H, 4.7; N, 21.9%).

6-Benzylamino-1,3-dimethylpyrimidine-2,4-dione.—Prepared according to the method of Bredereck (1960). To a solution of benzylamine (10 g) in ethanol (60 ml) was added 6-chloro-1,3-dimethyluracil (7.7 g) and the mixture heated under reflux (1.5 h). The residue obtained on evaporation was extracted with water (600 ml) portionwise and chilled giving colourless needles of 6-benzylamino-1,3-dimethylpyrimidine-2,4-dione (7 g), m.p. 148-149° (Bredereck 1960: 149°).

6-Benzylamino-5-bromo-1,3-dimethylpyrimidine-2,4-dione.—6-Benzylamino-1,3-dimethylpyrimidine-2,4-dione (10 g) and anhydrous sodium acetate (7.5 g) in cold acetic acid (1250 ml) (ice bath) were stirred and bromine (7 g) dissolved in acetic acid (50 ml) added dropwise. At the end of the addition, the reaction mixture was diluted with sodium acetate (5 g) in ice water (1 ℓ) causing 6-benzylamino-5-bromo-1,3-dimethylpyrimidine-2,4-dione to precipitate. Recrystallisation from carbon tetrachloride gave the required product, m.p. 135-136° (Bredereck 1960: 137°).

Amination attempts with 6-Benzylamino-5-bromo-1,3-dimethylpyrimidine-2,4-dione.—(a) With aqueous ammonia: The 5-bromopyrimidine (0.2 g) in aqueous ammonia solution (25% w/v) was heated under reflux for 4 h. On cooling 6-benzylamino-1,3-dimethylpyrimidine-2,4-dione (0.12 g) (i.r., n.m.r.) was obtained.

(b) With alcoholic ammonia: Under reflux conditions (4 h) 6-benzylamino-5-bromo-1,3-dimethylpyrimidine-2,4-dione (0.2 g) in saturated ethanolic ammonia was converted into 8-phenyltheophylline (0.1 g).

9-Benzyltheophylline.—Using the procedure of Blicke and Schaaf (1956) 5,6-diamino-1,3-dimethylpyrimidine-2,4-dione (7.6 g) was condensed with benzyl isothiocyanate (6.6 g) and the resulting 5-thioureidopyrimidine cyclised to 9-benzyltheophylline-8-thione by heating under reflux

in hydrochloric acid. This product was obtained crystalline (4.2 g) from acetic acid. On desulphurisation of the 8-thiopurine (1.8 g) with nitrous acid and then raising the pH of the solution to 8 with aqueous ammonia the required 9-benzyltheophylline (0.95 g) was obtained as crystals, m.p. 170-171° (Blicke and Schaaf, 1956: 167-169°) from butan-2-ol.

9-Benzyl-8-benzylthiotheophylline.—A solution of 9-benzyltheophylline-8-thione (0.5 g) in 5N sodium hydroxide (10 ml) was treated with benzyl bromide (0.4 ml), with stirring. After one hour the product was filtered off and recrystallised from methanol to give 9-benzyl-8-benzylthiotheophylline (0.44 g), m.p. 179-181° (Found: C, 64.2; H, 5.4; N, 14.1. $C_{21}H_{20}N_4O_2S$ requires: C, 64.2; H, 5.1; N, 14.2%).

Action of Benzyl bromide on 9-Benzyltheophylline.—When 9-benzyltheophylline (0.2 g) and benzyl bromide (0.15 g) in dimethylformamide (5 ml) were heated under reflux for 3 h and cooled, no product was obtained. To the solution was added petroleum ether (b.p.60-80°; 10 ml) and the mixture left for 24 h. The resulting crystalline precipitate was shown to be 7-benzyltheophylline (0.1 g) by comparison (m.p., i.r., and n.m.r.) with authentic material.

Action of Heat on 9-Benzyltheophylline hydrobromide.— The hydrobromide salt was prepared by evaporating to dryness a solution of 9-benzyltheophylline (0.2 g) in hydrobromic acid (48%, 3 ml). After leaving the salt in dimethylformamide (5 ml) for 2 h under reflux the mixture was concentrated and the residue taken up in a little water. On adjustment to pH 8 with ammonia solution a crystalline precipitate (0.1 g) of 7-benzyltheophylline (m.p., i.r., n.m.r., and t.l.c.) was obtained. A small amount of the unchanged 9-benzyl isomer was recovered on evaporation of the filtrate.

When the experiment was repeated using 9-benzyl-theophylline itself no rearrangement occurred, only the starting material was recovered.

B. 7,8-DIMETHYL-2-METHYLTHIOPURINE AND INTERMEDIATES

6-Amino-5-(N-methyl)acetamido-2-methylthiopyrimidine.—6-Amino-5-methylamino-2-methylthiopyrimidine (0.5 g) in ethanol (5 ml) was treated with acetic anhydride and kept at room temperature for 2 h. After evaporation the residue was recrystallised from ethanol giving 6-amino-5-(N-methyl)acetamido-2-methylthiopyrimidine (0.27 g), m.p. 250-251° (Found: C, 45.8; H, 5.5; N, 26.5. $C_8H_{12}N_4OS$ requires: C, 45.3; H, 5.7; N, 26.4%).

7,8-Dimethyl-2-methylthiopurine.—6-Amino-5-(N-methyl)acetamido-2-methylthiopyrimidine (0.25 g) was taken up in dimethylformamide (5 ml) containing anhydrous potassium carbonate (0.15 g) and heated under reflux for 2 h. The residue remaining after evaporation in vacuo was recrystallised from ethanol giving 7,8-dimethyl-2-methylthiopurine (0.1 g), m.p. 197-198° (Found: C, 49.8; H, 5.4; N, 28.8. $C_8H_{10}N_4S$ requires: C, 49.5; H, 5.2; N, 28.8%).

C. 2-CYCLOALKYLAMINO-6,9-DIMETHYLPURINES AND INTERMEDIATES

2-Chloro-4-methyl-6-methylamino-5-nitropyrimidine.—6-Methyl-5-nitrouracil, prepared by the literature method (Robins, 1956), on treatment with phosphorus oxychloride gave 2,4-dichloro-6-methyl-5-nitropyrimidine (Albert et al., 1954). Replacement of one of the chlorine atoms with methylamine provided the required 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine (Brown et al., 1966).

4-Methyl-6-methylamino-2-(morpholin-4-yl)-5-nitropyrimidine.—Ethanolic morpholine (7.80 g) was added slowly to a stirred ice-cold suspension of 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine (7.00 g) in ethanol (15 ml). During the addition of morpholine, the suspension became very thick, and ethanol (50 ml) was added. The contents of the flask were stirred at 25° for 2 h and then chilled, the solid was filtered off and recrystallised from methanol giving 4-methyl-6-methylamino-2-(morpholin-4-yl)-5-nitropyrimidine (6 g), m.p. 194-195° (Found: C, 47.6; H, 5.8; N, 27.7. $C_{10}H_{15}N_5O_3$ requires: C, 47.4; H, 6.00; N, 27.7%).

4-Methyl-6-methylamino-5-nitro-2-(piperidin-1-yl) pyrimidine.—Ethanolic piperidine (4.20 g, 2.5 mole) similarly with 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine (4 g) gave 4-methyl-6-methylamino-5-nitro-2-(piperidin-1-yl)pyrimidine (3.5 g), m.p. 166-168° (from petroleum ether, b.p. 60-80°)(Found: C, 52.1; H, 7.0; N, 27.7. $C_{11}H_{17}N_5O_2$ requires: C, 52.6; H, 6.8; N, 27.9%).

4-Methyl-6-methylamino-5-nitro-2-(pyrrolidin-1-yl) pyrimidine.—With ethanolic pyrrolidine (4.40 g) the above conditions gave 4-methyl-6-methylamino-5-nitro-2-(pyrrolidin-1-yl)pyrimidine (4.4 g), m.p. 200-202° (from petroleum ether, b.p. 60-80°)(Found: C, 50.7; H, 6.4; N, 29.6. $C_{10}H_{15}N_5O_2$ requires C, 50.6; H, 6.4; N, 29.5%).

5-Amino-4-methyl-6-methylamino-2-(morpholin-4-yl) pyrimidine.—A solution of 4-methyl-6-methylamino-2-(morpholin-4-yl)-5-nitropyrimidine (2.5 g) in ethanol containing Raney nickel was hydrogenated to completion, 5-amino-4-methyl-6-methylamino-2-(morpholin-4-yl) pyrimidine (2.1 g) was obtained after the removal of the catalyst and evaporation of ethanol. Recrystallisation from petroleum ether (b.p. 60-80°) gave the pure aminopyrimidine, m.p. 115-117° (Found: C, 53.7; H, 7.6; N, 31.6. $C_{10}H_{17}N_5O$ requires: C, 53.8; H, 7.7; N, 31.4%).

5-Amino-4-methyl-6-methylamino-2-(piperidin-1-yl) pyrimidine.—An ethanolic solution of 4-methyl-6-methylamino-5-nitro-2-(piperidin-1-yl)pyrimidine (2.5 g) was likewise reduced to give 5-amino-4-methyl-6-methylamino-2-(piperidin-1-yl)pyrimidine (1.69 g), m.p. 120-121° after recrystallisation from petroleum ether (b.p. 60-80°)(Found: C, 59.7; H, 8.5; N, 32.1. $C_{11}H_{19}N_5$ requires: C, 59.7; H, 8.6; N, 31.7%).

5-Amino-4-methyl-6-methylamino-2-(pyrrolidin-1-yl) pyrimidine.—4-Methyl-6-methylamino-5-nitro-2-(pyrrolidin-1-yl)pyrimidine (4 g) similarly gave 5-amino-4-methyl-6-methylamino-2-(pyrrolidin-1-yl) pyrimidine (2.7 g), m. p. 127-128° (from petroleum ether, b.p. 60-80°)(Found: C, 57.8; H, 8.1; N, 33.3. $C_{10}H_{17}N_5$ requires: C, 57.9; H, 8.3; N, 33.8%).

6,9-Dimethyl-2-(morpholin-4-yl)purine.—5-Amino-4-methyl-6-methylamino-2-(morpholin-4-yl)pyrimidine (1 g) and formic acid (98%, 15 ml) were heated under reflux for 2 h and the solution then evaporated to dryness. Crystallisation of the residue from petroleum ether (b.p. 60-80°) gave 6,9-dimethyl-2-(morpholin-4-yl)purine (0.55 g), m.p. 151-153° (Found: C, 56.3; H, 6.3; N, 29.9. $C_{11}H_{15}N_5O$ requires: C, 56.6; H, 6.5; N, 30.0%). pK_a 3.95±0.05 (anal. λ 240 nm).

In like manner were prepared from 1 g of the appropriate 2-alkylamino-5-amino-4-methyl-6-methylaminopyrimidine:

6,9-Dimethyl-2-(piperidin-1-yl)purine (0.55 g), m.p. 122-123° (from petroleum ether, b.p. 60-80°) (Found: C, 62.2; H, 7.1; N, 30.7. $C_{12}H_{17}N_5$ requires: C, 62.3; H, 7.4; N, 30.3%) pK_a 4.66±0.04 (anal λ 240 nm) and 6,9-Dimethyl-2-(pyrrolidin-1-yl)purine (0.5 g), m.p. 90-92° (from petroleum ether, b.p. 60-80°) (Found: C, 59.5; H, 6.8; N, 31.7. $C_{11}H_{15}N_5O.0.25H_2O$ requires: C, 59.4; H, 7.0; N, 31.5%).

2-(Hexahydroazepin-1-yl)-6,9-dimethylpurine.— Similar procedures with hexahydroazepine in place of pyrrolidine gave successively 2-(hexahydroazepin-1-yl)-4-methyl-6-methylamino-5-nitropyrimidine (67%), m.p. 115-117° (Found: C, 54.6; H, 7.3; N, 26.5. $C_{12}H_{19}N_5O_2$ requires: C, 54.4; H, 7.2; N, 26.4%); and the hygroscopic 5-amino-2-(hexahydroazepin-1-yl)-4-methyl-6-methylaminopyrimidine (94%), m.p. 82-84° (Found: C, 60.2; H, 9.0; N, 29.1. $C_{12}H_{21}N_5.0.25H_2O$ requires: C, 60.1; H, 9.0; N, 29.1%); and the 2-(hexahydroazepin-1-yl)-6,9-dimethylpurine (48%), m.p. 62-64° (Found: C, 63.5; H, 7.8; N, 28.9. $C_{13}H_{19}N_5$ requires: C, 63.6; H, 7.8; N, 28.6%).

6,9-Dimethyl-2-(octahydroazocin-1-yl)purine.—

In a like manner octahydroazocine gave 4-methyl-6-methylamino-5-nitro-2-(octahydroazocin-1-yl)pyrimidine (58%), m.p. 107-109° (Found: C, 55.4; H, 7.8; N, 24.6. $C_{13}H_{21}N_5O_2$ requires: C, 55.9; H, 7.6; N, 25.1%) and on reduction, 5-amino-4-methyl-6-methylamino-2-(octahydroazocin-1-yl)pyrimidine (72%), m.p. 82-84° was obtained (Found: N, 26.2. $C_{13}H_{23}N_5.H_2O$ requires: N, 26.2%). This aminopyrimidine (1 g) and 98% formic acid (15 ml) when boiled under reflux for 4 h and evaporated gave the crude formyl derivative which cyclised on heating for 40 min in formamide containing a drop of concentrated hydrochloric acid. Refrigeration gave the octahydroazocinylpurine (34%), m.p. 79-81° (from light petroleum) (Found: C, 63.8; H, 7.9; N, 26.8. $C_{14}H_{21}N_5.0.25\ H_2O$ requires: C, 63.7; H, 8.2; N, 26.6%).

D. 6-AMINO-8-ETHOXYPURINES AND INTERMEDIATES

4-Amino-6-chloro-5-nitropyrimidine.—4,6-Dihydroxypyrimidine, obtained according to the method of Kenner et al. (1943) was nitrated, using the literature conditions (Boon et al., 1951), and the resulting 4,6-dihydroxy-5-nitropyrimidine then chlorinated (Boon, 1951) to give 4,6-dichloro-5-nitropyrimidine. By following the procedure of Segal et al. (1959) the monoamination of 4,6-dichloro-5-nitropyrimidine was carried out with methanolic ammonia in tetrahydrofuran containing sodium hydrogen carbonate. The required 4-amino-6-chloro-5-nitropyrimidine was obtained in good yield (70%) with a m.p. 155-159° (from benzene).

General procedure for the aminolysis of 4-amino-6-chloro-5-nitropyrimidine.—One gram of 4-amino-6-chloro-5-nitropyrimidine was added to n-butanol (20 ml) containing the appropriate amine (2 g) as reported by Daly et al. (1956). After heating under reflux for 1.5 h the solution was cooled, and the product filtered off and recrystallised.

Following this procedure 4-amino-6-anilino-5-nitropyrimidine, m.p. 211-213° and 4-amino-6-benzylamino-5-nitropyrimidine, m.p. 195-197° were prepared.

4-Chloro-6-methylamino-5-nitropyrimidine.—Following the method of Brown (1957) 4,6-dichloro-5-nitropyrimidine was treated with aqueous methylamine (25% w/v) in dioxan at 15-20°, to give 4-chloro-6-methylamino-5-nitropyrimidine, m.p. 147-148° (reported 147-148°).

4-Amino-6-methylamino-5-nitropyrimidine.—4-Chloro-6-methylamino-5-nitropyrimidine (2 g) when treated with an excess of methanolic ammonia produced 4-amino-6-methylamino-5-nitropyrimidine (1.5 g), m.p. 244-246° (reported 242-245°).

General procedure for reduction of 4-amino-5-nitro-6-alkylaminopyrimidine.—The pyrimidine (2 g) in methanol (150 ml) was hydrogenated to completion over Raney nickel at room temperature. The triamines obtained were used immediately for condensation with the orthocarbonates.

Condensation of 4,5-diamino-6-anilinopyrimidine with tetraethoxymethane.—4,5-Diamino-6-anilinopyrimidine (0.2 g) and an excess of tetraethoxymethane were heated under reflux for 36 h. The reaction mixture was concentrated and refrigerated, and the resulting solid treated with 1N sodium hydroxide and filtered. The filtrate upon acidification gave no product.

The solid was dissolved in ethanol and the solution treated with ether giving a dark brown solid (0.08 g), m.p. 170-172° (Found: C, 61.4; H, 5.6. $C_{13}H_{13}N_5O$ requires: C, 61.2; H, 5.1%). The ^1H n.m.r. was quite consistent with this product being 8-ethoxy-9-phenyladenine δ[$(CD)_2SO$] 1.36(t, CH_2CH_3), 4.56(q, CH_2CH_3), 6.83(s, NH), 7.5(s, C_6H_5), and 8.0(s, 2-H).

Condensation of 4,5-diamino-6-benzylaminopyrimidine with tetraethoxymethane.—4,5-Diamino-6-benzylamino-pyrimidine (0.2 g) was heated (12 h) as above with an excess of tetraethoxymethane. After filtering and concentration the solution was refrigerated affording crystals, m.p. 124-126°. The n.m.r. and analysis indicated it to be 4-amino-6-benzylamino-5-diethoxy-methyleneaminopyrimidine (Found: C, 60.0; H, 6.6; N, 21.8. $C_{16}H_{21}N_5O_2$ requires: C, 60.9; H, 6.7; N, 22.2%).

Condensation of 4,5-diamino-6-methylaminopyrimidine with tetraethoxymethane.—Heating (24 h) 4,5-diamino-6-methylaminopyrimidine (0.2 g) and an excess of tetra-ethoxymethane gave a solid which was digested with alkali and filtered.

The solid upon recrystallisation from ethanol gave 8-ethoxy-9-methyladenine (0.08 g, 32%), m.p. 230-232° (Found: C, 49.4; H, 6.1. $C_8H_{11}N_5O$ requires: C, 49.7; H, 5.7%). The ^1H n.m.r. spectrum was in agreement with the structure of this derivative. $\delta[(CD)_3SO]$ 1.40(t, CH_2CH_3), 3.42(s, NMe), 4.52(q, CH_2CH_3), 6.65(s, NH) and 8.0(s, 2-H).

The alkaline filtrate upon acidification gave a product presumed to be 8-ethoxy-6-methylaminopurine (0.05 g, 18%), m.p. 209-211° (Found: C, 44.9; H, 5.7. $C_8H_{13}N_5O_2$ requires: C, 45.5; H, 6.2%).

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List of Compounds

Page No.

PYRIMIDINES

OTHER DERIVATIVES

Publications

The following papers are based upon or include work described in this thesis:

1. Purine Studies. XVII.
   The Synthesis of 2-Substituted 6,9-Di- and 6,8,9-Tri-methylpurines as Amplifiers of Phleomycin.
   K. Bhushan, D.J. Brown, J.H. Lister, L.G. Stephanson and F. Yoneda
   Austral. J. Chem., 1975, 28, 2553.

2. Purine Studies. XVIII.
   The *C*- and *N*-Alkylation of Theophylline and Derivatives.
   K. Bhushan and J.H. Lister
   Austral. J. Chem., 1976, 29 (~~in press~~ enclosed).

Aust. J. Chem., 1975, **28**, 2553–9

Purine Studies. XVII*
The Synthesis of 2-Substituted 6,9-Di- and 6,8,9-Tri-methylpurines as Amplifiers of Phleomycin

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Abstract

2-(6′,8′,9′-Trimethylpurin-2′-ylthio)acetamide (1e) and analogous *N*-substituted acetamides are prepared by treatment of 6,8,9-trimethylpurine-2-thione (3a) with an appropriate 2-chloroacetamide. 6,9-Dimethyl-2-(piperidin-1′-yl)purine (1n) and some 2-polymethyleneamino homologues are made by initial amination of 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine (2b) followed by reduction of the nitro group and final cyclization with formic acid. Such purines enhance the lethal effect of phleomycin on *Escherichia coli* cultures.

Previous papers have described how the antibacterial activity of phleomycin towards *Escherichia coli* is potentiated considerably *in vitro* by the addition of certain purines.[1–4] However, if such a combination is to be useful *in vivo*, for example against a urinary tract infection,[1] oxidative metabolism of the purine must be minimized by appropriate substitution; at the same time, this must be consistent with the maintenance of high activity.

Of some seventy purines so far tested, most of those with high *in vitro* activity bore, not only *C*-alkyl groups to prevent hydroxylation, but also an alkylthio or dimethylamino substituent. Although the alkylthiopurine (1a) underwent extensive metabolism in mice to give the corresponding (inactive) sulphoxide (1b),[2] such oxidation was decreased by the addition of an amide grouping to the alkylthio substituent.[5] Thus neither the active amide (1c) nor the dimethylaminopurines underwent appreciable oxidation at their respective functional groupings.

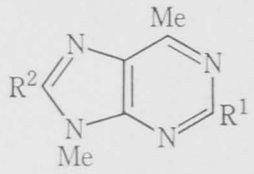
To develop this lead, we now record the preparation and activity of some 6,9-dimethyl- and 6,8,9-trimethyl-purines bearing at the 2-position either a substituted carbamoylmethylthio group or a polymethyleneamino group.

5-Amino-2-chloro-4-methyl-6-methylaminopyrimidine (2a), prepared by an improved route from 6-methyluracil via the nitropyrimidine (2b), was boiled with

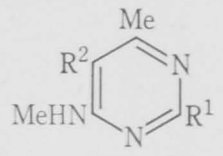
* Part XVI, *J. Chem. Soc., Perkin Trans. 2*, 1975, paper 5/699.

[1] Grigg, G. W., Edwards, M. J., and Brown, D. J., *J. Bacteriol.*, 1971, **107**, 599; Grigg, G. W., *J. Gen. Microbiol.*, 1972, **70**, 221.
[2] Brown, D. J., Jones, R. L., Angyal, A. M., and Grigg, G. W., *J. Chem. Soc., Perkin Trans. 1*, 1972, 1819.
[3] Badger, R. J., Brown, D. J., and Lister, J. H., *J. Chem. Soc., Perkin Trans. 1*, 1974, 152.
[4] Angyal, A. M., Grigg, G. W., Badger, R. J., Brown, D. J., and Lister, J. H., *J. Gen. Microbiol.*, 1974, **85**, 163.
[5] Brown, D. J., and Stephanson, L. G., *Aust. J. Chem.*, 1974, **27**, 1371.

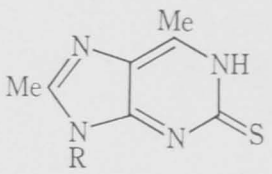
acetic anhydride to give directly the chloropurine (1d). This reacted slowly with sodium hydrogen sulphide to give the thione (3a) previously made[2] by a less convenient route; treatment of the thione with chloroacetamide in aqueous sodium hydrogen carbonate gave the (purinylthio)acetamide (1e). The similar use of appropriate *N*-substituted chloroacetamides gave the analogues (1f–k); another analogue (1l) was made from the same purinethione and 2-chloropropionamide and



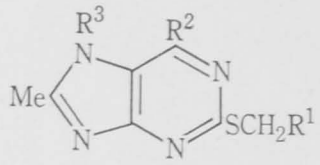
	R^1	R^2
(1a)	SMe	H
(1b)	SOMe	H
(1c)	SCH_2CONH_2	H
(1d)	Cl	Me
(1e)	SCH_2CONH_2	Me
(1f)	SCH_2CONMe_2	Me
(1g)	SCH_2CONEt_2	Me
(1h)	$SCH_2CONHMe$	Me
(1i)	$SCH_2CON[(CH_2CH_2)_2O]$	Me
(1j)	$SCH_2CONHCH_2CH_2OH$	Me
(1k)	$SCH_2CONHPh$	Me
(1l)	$SCHMeCONH_2$	Me
(1m)	SPr^i	Me
(1n)	$N(CH_2)_5$	H
(1o)	$N(CH_2)_4$	H
(1p)	$N(CH_2)_6$	H
(1q)	$N(CH_2)_7$	H
(1r)	$N[(CH_2CH_2)_2O]$	Me
(1s)	$N(CH_2)_5$	Me



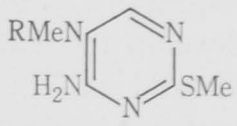
	R^1	R^2
(2a)	Cl	NH_2
(2b)	Cl	NO_2
(2c)	$SCHMeCONH_2$	NHAc
(2d)	$SCHMeCONH_2$	NH_2
(2e)	$N(CH_2)_5$	NO_2
(2f)	$N(CH_2)_5$	NH_2
(2g)	$N(CH_2)_4$	NO_2
(2h)	$N(CH_2)_4$	NH_2
(2i)	$N(CH_2)_6$	NO_2
(2j)	$N(CH_2)_6$	NH_2
(2k)	$N(CH_2)_7$	NO_2
(2l)	$N(CH_2)_7$	NH_2
(2m)	$N[(CH_2CH_2)_2O]$	NO_2
(2n)	$N[(CH_2CH_2)_2O]$	NH_2
(2o)	$N(CH_2)_5$	NHAc



	R
(3a)	Me
(3b)	H



	R^1	R^2	R^3
(4a)	$CONH_2$	Me	H
(4b)	H	H	Me



	R
(5a)	H
(5b)	Ac

also by cyclization of the *N*-(pyrimidin-5′-yl)acetamide (2c), prepared from 5-amino-4-methyl-6-methylaminopyrimidine-2-thione[6] via its derivative (2d). In addition, 2-(6′,8′-dimethylpurin-2′-ylthio)acetamide (4a) was made from the known[2] thione (3b); the purine (1m), from the thione (3a); and 7,8-dimethyl-2-methylthiopurine (4b), from the known[7] diamine (5a) via its acetyl derivative (5b).

[6] Brown, D. J., Ford, P. W., and Tratt, K. H., *J. Chem. Soc., C*, 1967, 1445.
[7] Brown, D. J., *J. Appl. Chem.*, 1955, **5**, 358.

Treatment of the chloronitropyrimidine (2b) with cold ethanolic piperidine gave the piperidinylpyrimidine (2e) which was reduced to the 5-amino analogue (2f). In boiling formic acid, this underwent acylation and subsequent cyclization to the dimethylpiperidinylpurine (1n). The homologous pyrrolidinyl-, hexahydroazepinyl-, and octahydroazocinyl-purines (1o–q) were made similarly by the sequences [(2b) → (2g) → (2h) → (1o)], [(2b) → (2i) → (2j) → (1p)], and [(2b) → (2k) → (2l) and (1q)] respectively; the morpholinylpurine (1r), via the pyrimidines (2m) and (2n); and the trimethylpiperidinylpurine (1s), by two routes: (*a*) directly from the chloropurine (1d) and (*b*) from the 5-aminopyrimidine (2f) via its acetyl derivative (2o).

Values for the biological activity of a selection of the above purines and for several unrelated derivatives[8,9] appear in Table 1. It is evident that some of the amides [(1e), (1f), (1i), (1j), (1l), (4a)] and polymethyleneamino derivatives [(1n), (1r), (1s)] have the expected high to very high *in vitro* activity. Thus they fulfil the first criterion for practical use as amplifiers of phleomycin against infections. Their performance in respect of the second criterion, viz. resistance to metabolic change, will be reported later.

Table 1. Activities of purines as phleomycin amplifiers

For definition of adjusted activity (A_{ad}) see ref. 4, which also explains why some values are printed in *italics*

Purine	Act.	A_{ad}	Purine	Act.	A_{ad}	Purine	Act.	A_{ad}
(1c)	++++	510[A]	(1j)	+++	145	(4a)	+++	265
(1d)	++	65	(1k)	+	*0·2*	7-Et-6-Me-2-SMe[B]	++	22
(1e)	+++	130	(1l)	++++	430	6-Me-2-SMe-8-CF_3[C]	+	0·2
(1f)	+++	290	(1m)	+	*2*	7-Et-6-Me-2-SMe-8-CF_3[C]	+	*0·1*
(1g)	++	20	(1n)	++++	*235*	9-Et-6-Me-2-SMe-8-CF_3[C]	+	*0·1*
(1h)	++	20	(1r)	+++	*40*	caffeine[A]	++	30
(1i)	+++	105	(1s)	+++	*90*	caffeine[A]	++	*11*

[A] From ref. 4. [B] Prep.: ref. 8. [C] Prep.: ref. 9.

Experimental

Analyses were done by the Australian National University Analytical Services Unit. The anhydrous compounds (1k), (1o), (1q), (2c), (2j), (2l) were homogeneous to t.l.c. and paper chromatography but proved to be too hygroscopic for satisfactory analyses as such: accordingly, each was equilibrated in the atmosphere for 12–24 h prior to analysis as the hydrated species described below. Melting points were uncorrected. Ionization constants were measured spectrometrically[10,11] at 20° without thermodynamic corrections. The n.m.r. spectra were measured in $CDCl_3$, except where indicated otherwise, at 35° and 60 MHz on a Varian T60A instrument with Me_4Si as an internal standard (chemical shifts in δ; J values in Hz); u.v. spectra were recorded in aqueous buffer on an SP 1800; and i.r. spectra were measured in Nujol mulls on an SP 200. The method for *in vitro* evaluation of purines as amplifiers of phleomycin against *E. coli B* has been described in detail.[4]

2-Chloro-6,8,9-trimethylpurine (1d)

Nitric acid (*d* 1·5; 125 ml) was added gradually during 2 h to a stirred solution of 6-methyluracil (100 g) in concentrated sulphuric acid (300 ml), maintained at 5–10°. After a further 2 h at 20–25°,

[8] Fenn, M. D., and Lister, J. H., *J. Chem. Soc., Perkin Trans. 1*, 1974, 1300.
[9] Fenn, M. D., and Lister, J. H., *J. Chem. Soc., Perkin Trans. 1*, 1975, 485.
[10] Albert, A., and Serjeant, E. P., 'Determination of Ionization Constants' (Chapman & Hall: London 1971).
[11] Perrin, D. D., *Aust. J. Chem.*, 1963, **16**, 572.

the mixture was poured onto ice (1 kg). The crystalline material was washed with water to give 6-methyl-5-nitrouracil (93%) (cf. lit.[12] 65%). This was converted[13] into 2,4-dichloro-6-methyl-5-nitropyrimidine which underwent methylamination[14] to 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine (2b) [recrystallization of the crude material from light petroleum left an insoluble by-product, identified as 4-methyl-2,6-bis(methylamino)-5-nitropyrimidine, m.p. 252° (from ethanol) (cf. lit.[15] 228 or 235°; different crystalline form?) (Found: C, 42·5; H, 5·5; N, 35·6. Calc. for $C_7H_{11}N_5O_2$: C, 42·6; H, 5·6; N, 35·5%)] and subsequent reduction[14] to 5-amino-2-chloro-4-methyl-6-methylaminopyrimidine (2a). This material (4·3 g) and acetic anhydride (15 ml) were boiled under reflux for 6 h. The residue from concentration under reduced pressure was extracted with boiling ether. Evaporation of the extracts and recrystallization of the residue from light petroleum–methanol gave the *chlorotrimethylpurine* (63%), m.p. 171–172° (Found: C, 48·9; H, 4·8; N, 28·6. $C_8H_9ClN_4$ requires C, 48·9; H, 4·6; N, 28·5%). pK_a 1·83±0·05 (anal. λ 286 nm); u.v. λ_{max} (log ε) at pH 7: 214 (4·37), *245* (3·60), 273 (4·03); at pH 0: 207 (4·39), 234 (3·62), 270 (4·03), *277* (3·97); n.m.r. (Me_2SO) 2·58 (s, 8-Me), 2·63 (s, 6-Me), 3·67 (s, 9-Me).

6,8,9-Trimethylpurine-2(1H)-thione (*3a*)

The above chloropurine (3 g) was heated under reflux with aqueous 2M sodium hydrogen sulphide (75 ml) for 20 h. The cooled solution was acidified with acetic acic and then evaporated to dryness. Crystallization of the residue from water gave the purinethione (52%), identified with authentic material[2] by i.r. and u.v. spectra.

2-(6′,8′,9′-Trimethylpurin-2′-ylthio)acetamide (*1e*)

The above purinethione (125 mg), 2-chloroacetamide (85 mg), sodium hydrogen carbonate (72 mg) and water (2·5 ml) were boiled under reflux for 90 min. Refrigeration of the filtered solution gave the (*trimethylpurinylthio)acetamide* (89%), m.p. 212–213° (from methanol) (Found: C, 47·5; H, 5·2; N, 27·8. $C_{10}H_{13}N_5OS$ requires C, 47·8; H, 5·2; N, 27·9%). M^+ 251; ν_{max} 1680 (C=O).

N-*Substituted 2-(6′,8′,9′-Trimethylpurin-2′-ylthio)acetamides*

The purinethione (0·97 g), 2-chloro-*N*,*N*-dimethylacetamide (0·61 g), sodium hydrogen carbonate (0·5 g) and water (20 ml) were heated under reflux for 1 h. The oily residue from evaporation under reduced pressure was extracted with chloroform. The dehydrated extract was evaporated to small bulk and diluted with ether to give N,N-*dimethyl-2-(6′,8′,9′-trimethylpurin-2′-ylthio)acetamide* (1f) (64%), m.p. 194° (from chloroform–ether) (Found: C, 51·8; H, 6·1; N, 25·1. $C_{12}H_{17}N_5OS$ requires C, 51·6; H, 6·1; N, 25·1%). ν_{max} 1644 (C=O); n.m.r. 2·58 (s, 8′-Me), 2·70 (s, 6′-Me), 2·99 (s, Me of NMe_2), 3·18 (s, Me of NMe_2), 3·70 (s, 9′-Me), 4·18 (s, CH_2).

The following were prepared similarly. 2-Chloro-*N*,*N*-diethylacetamide gave N,N-*diethyl-2-(6′,8′,9′-trimethylpurin-2′-ylthio)acetamide* (1g) (57%), m.p. 100° (Found: C, 54·5; H, 6·8; N, 22·9. $C_{14}H_{21}N_5OS$ requires C, 54·7; H, 6·9; N, 22·8%). ν_{max} 1641 (C=O); n.m.r. 1·13 (t, *J* 7, Me of Et), 1·28 (t, *J* 7, Me of Et), 2·57 (s, 8′-Me), 2·68 (s, 6′-Me), 3·43 (q, *J* 7, CH_2 of Et), 3·50 (q, *J* 7, CH_2 of Et), 3·67 (s, 9′-Me), 4·14 (s, SCH_2). 2-Chloro-*N*-methylacetamide gave N-*methyl-2-(6′,8′,9′-trimethylpurin-2′-ylthio)acetamide* (1h) (64%), m.p. 197–198° (Found: C, 49·8; H, 5·7; N, 26·7. $C_{11}H_{15}N_5OS$ requires C, 49·8; H, 5·7; N, 26·4%). ν_{max} 1664 (C=O); n.m.r. 2·60 (s, 8′-Me), 2·71 (s, 6′-Me), 2·76 (d, *J* 5, Me of NHMe), 3·69 (s, 9′-Me), 3·88 (s, CH_2), 7·07 (br s, NH). *N*-(Chloroacetyl)morpholine gave *4-[2′-(6″,7″,8″-trimethylpurin-2″-ylthio)acetyl]morpholine* (1i) (75%), m.p. 169° (Found: C, 52·1; H, 5·9; N, 21·7. $C_{14}H_{19}N_5O_2S$ requires C, 52·3; H, 6·0; N, 21·8%). ν_{max} 1641 (C=O); n.m.r. 2·58 (s, 8″-Me), 2·70 (s, 6″-Me), 3·64 [s, $(CH_2)_4$ of morpholine], 3·68 (s, 9″-Me), 4·17 (s, CH_2).

2-Chloro-*N*-(2-hydroxyethyl)acetamide gave N-*(2′-hydroxyethyl)-2-(6′,8′,9′-trimethylpurin-2′-ylthio)acetamide* (1j) (41%), m.p. 227–229° (Found: C, 48·9; H, 5·8; N, 23·5. $C_{12}H_{17}N_5O_2S$

[12] Gabriel, S., and Colman, J., *Ber. Deut. Chem. Ges.*, 1901, **34**, 1234.
[13] Albert, A., Brown, D. J., and Wood, H. C. S., *J. Chem. Soc.*, 1954, 3832.
[14] Brown, D. J., England, B. T., and Lyall, J. M., *J. Chem. Soc., C*, 1966, 226.
[15] Goldner, H., and Carstens, E., *J. Prakt. Chem.*, 1961, [4] **12**, 242; Cohen, S., and Vincze, A., *Israel J. Chem.*, 1964, **2**, 1.

requires C, 48·8; H, 5·8; N, 23·7%). ν_{max} 1648 (C=O); n.m.r. (Me_2SO) 2·51 (s, 8′-Me), 2·56 (s, 6′-Me), 3·27 (m, CH_2CH_2), 3·65 (s, 9′-Me), 3·87 (s, SCH_2), 8·08 (br s, NH).

The thiopurine (0·5 g), α-chloroacetanilide (0·46 g), sodium hydrogen carbonate (0·25 g) and water (10 ml) were boiled under reflux for 2 h. The cooled solution deposited *2-(6′,8′,9′-trimethylpurin-2′-ylthio)acetanilide* (1k) (46%), m.p. 195° (from methanol) (Found: C, 55·8; H, 5·5; N, 20·0. $C_{16}H_{17}N_5OS,H_2O$ requires C, 55·6; H, 5·5; N, 20·3%). ν_{max} 1668 (C=O); n.m.r. (Me_2SO) 2·51 (s, 8′-Me), 2·57 (s, 6′-Me), 3·62 (s, 9′-Me), 4·08 (s, CH_2), 7·4 (br m, 5H of Ph), 10·23 (br s, NH).

2-(6′,8′,9′-Trimethylpurin-2′-ylthio)propionamide (*1l*)

(A) 6,8,9-Trimethylpurine-2-thione (195 mg), 2-chloropropionamide (130 mg), sodium hydrogen carbonate (100 mg) and water (2·0 ml) were heated under reflux for 30 min. Refrigeration gave the *(trimethylpurinylthio)propionamide* (31%), m.p. 228–229° (from water) (Found: C, 49·1; H, 5·8; N, 25·8. $C_{11}H_{15}N_5OS$ requires C, 49·0; H, 5·8; N, 26·0%). M^+ 265; ν_{max} 1700 (C=O).

(B) 5-Amino-4-methyl-6-methylaminopyrimidine-2-thione[6] (1·7 g), 2-chloropropionamide (1·08 g), sodium hydrogen carbonate (0·93 g) and water (20 ml) were heated under reflux for 30 min. Then chloropropionamide (0·35 g) in water (30 ml) was added and heating was continued for a further 40 min. The chilled mixture deposited *2-(5′-amino-4′-methyl-6′-methylaminopyrimidin-2′-ylthio)propionamide* (2d) (65%), m.p. 201–203° (from water) (Found: C, 44·6; H, 6·2; N, 28·9. $C_9H_{15}N_5OS$ requires C, 44·8; H, 6·2; N, 29·1%). M^+ 241; ν_{max} 1670 (C=O). This material (120 mg), ethanol (10 ml), and acetic anhydride (60 mg) were boiled under reflux for 10 min. Then more anhydride (30 mg) was added and heating was continued for a further 10 min. The residue from evaporation was washed with a little water to give *2-(5′-acetamido-4′-methyl-6′-methylaminopyrimidin-2′-ylthio)propionamide* (2c) (90%), m.p. 205–206° (from ethanol–light petroleum) (Found: C, 44·5; H, 6·0; N, 23·6. $C_{11}H_{17}N_5O_2S,0{\cdot}75H_2O$ requires C, 44·5; H, 6·3; N, 23·6%). M^+ 283; ν_{max} 1610, 1680 (C=O). The acetamido derivative (30 mg), potassium carbonate (40 mg) and dimethylformamide (5 ml) were stirred at 120° for 7 h. The residue from evaporation in a vacuum was washed with a little cold water to give the (trimethylpurinylthio)propionamide (37%), identified with that from (A) by mixed m.p.

2-(6′,8′-Dimethylpurin-2′-ylthio)acetamide (*4a*)

6,8-Dimethylpurine-2-thione (3b)[2] was treated with chloroacetamide as was its trimethyl homologue above. The resulting *(dimethylpurinylthio)acetamide* (25%) had m.p. 264–267° (dec.) (from methanol–ether) (Found: C, 45·4; H, 4·7; N, 29·3. $C_9H_{11}N_5OS$ requires C, 45·6; H, 4·7; N, 29·5%). M^+ 295; ν_{max} 1690 (C=O).

2-Isopropylthio-6,8,9-trimethylpurine (*1m*)

The trimethylpurinethione (100 mg) and isopropyl iodide (170 mg) were stirred in aqueous 5% sodium hydroxide (10 ml) at 25°. After 25, 50 and 75 h, further portions (170 mg) of isopropyl iodide were added; at 100 h, the product was filtered off and recrystallized from water (charcoal) to give the *isopropylthiotrimethylpurine* (55%), m.p. 112° (Found: C, 56·2; H, 6·6; N, 24·1. $C_{11}H_{16}N_4S$ requires C, 55·9; H, 6·8; N, 23·7%). M^+ 236.

7,8-Dimethyl-2-methylthiopurine (*4b*)

Acetic anhydride (3 ml) was added to a solution of 4-amino-5-methylamino-2-methylthiopyrimidine (5a)[7] (0·5 g) in ethanol (5 ml). After 2 h at 25°, the mixture was evaporated to give N-*(4′-amino-2′-methylthiopyrimidin-5′-yl)*-N-*methylacetamide* (5b) (60%), m.p. 250–251° (from ethanol) (Found: C, 45·8; H, 5·5; N, 26·5. $C_8H_{12}N_4OS$ requires C, 45·3; H, 5·7; N, 26·4%). This pyrimidine (0·25 g), dimethylformamide (5 ml) and anhydrous potassium carbonate (0·15 g) were heated under reflux for 90 min. The cooled mixture was filtered, diluted threefold with water and evaporated to give the *dimethylmethylthiopurine* (32%), m.p. 197–198° (from ethanol) (Found: C, 49·8; H, 5·4; N, 28·8. $C_8H_{10}N_4S$ requires C, 49·5; H, 5·2; N, 28·8%). (It is evident that an isomeric *N*,8-dimethyl-2-methylthiopurine of m.p. 158–159° was incorrectly suspected[16] of having the above structure.)

[16] Brown, D. J., and Ford, P. W., *J. Chem. Soc.*, *C*, 1969, 2620.

6,9-Dimethyl-2-(piperidin-1′-yl)purine (*1n*)

Piperidine (5·1 g) in ethanol (10 ml) was added slowly to a stirred suspension of the above 2-chloro-4-methyl-6-methylamino-5-nitropyrimidine (2b) (4·07 g) in ethanol (10 ml) maintained at *c.* 5°. After a further 2 h at 20–25°, *4-methyl-6-methylamino-5-nitro-2-(piperidin-1′-yl)pyrimidine* (2e) (92%) was filtered off. Recrystallized from ethanol and then light petroleum, it had m.p. 166–168° (Found: C, 52·1; H, 7·0; N, 27·7. $C_{11}H_{17}N_5O_2$ requires C, 52·6; H, 6·8; N, 27·9%). This nitro compound (4·65 g) was hydrogenated at atmospheric pressure in methanol (600 ml) at 20° over Raney nickel. Evaporation of the filtered solution gave *5-amino-4-methyl-6-methylamino-2-(piperidin-1′-yl)pyrimidine* (2f) (73%), m.p. 121–124° (from light petroleum) (Found: C, 59·7; H, 8·5; N, 32·1. $C_{11}H_{19}N_5$ requires C, 59·7; H, 8·6; N, 31·7%). This 5-aminopyrimidine (1 g) and formic acid (98%; 15 ml) were heated under reflux for 2 h. The residue from evaporation crystallized from light petroleum to give the *dimethylpiperidinylpurine* (53%), m.p. 122–123° (Found: C, 62·2; H, 7·1; N, 30·7. $C_{12}H_{17}N_5$ requires C, 62·3; H, 7·4; N, 30·3%). pK_a 4·66±0·04 (anal. λ 240 nm).

6,9-Dimethyl-2-(pyrrolidin-1′-yl)purine (*1o*)

2-Chloro-4-methyl-6-methylamino-5-nitropyrimidine (4·4 g) in ethanol (150 ml) was treated with pyrrolidine (5 g) (as for the piperidinyl homologue above) to give *4-methyl-6-methylamino-5-nitro-2-(pyrrolidin-1′-yl)pyrimidine* (2 g) (86%), m.p. 200–202° (from light petroleum) (Found: C, 50·7; H, 6·4; N, 29·6. $C_{10}H_{15}N_5O_2$ requires C, 50·6; H, 6·4; N, 29·5%) which underwent hydrogenation to yield *5-amino-4-methyl-6-methylamino-2-(pyrrolidin-1′-yl)pyrimidine* (2h) (78%), m.p. 127–128° (from light petroleum) (Found: C, 57·8; H, 8·1; N, 33·3. $C_{10}H_{17}N_5$ requires C, 57·9; H, 8·3; N, 33·8%) and subsequent cyclization with formic acid to the *dimethylpyrrolidinylpurine* (45%), m.p. 90–92° (from light petroleum) (Found: C, 59·5; H, 6·8; N, 31·7. $C_{11}H_{15}N_5$,0·25H_2O requires C, 59·4; H, 7·0; N, 31·5%).

2-(Hexahydroazepin-1′-yl)-6,9-dimethylpurine (*1p*)

Similar procedures with hexahydroazepine in place of pyrrolidine gave successively *2-(hexahydroazepin-1′-yl)-4-methyl-6-methylamino-5-nitropyrimidine* (2i) (67%), m.p. 115–117° (Found: C, 54·6; H, 7·3; N, 26·5. $C_{12}H_{19}N_5O_2$ requires C, 54·4; H, 7·2; N, 26·4%); the hygroscopic *5-amino-2-(hexahydroazepin-1′-yl)-4-methyl-6-methylaminopyrimidine* (2j) (94%), m.p. 82–84° (Found: C, 60·2; H, 9·0; N, 29·1. $C_{12}H_{21}N_5$,0·25H_2O requires C, 60·1; H, 9·0; N, 29·1%); and the *hexahydroazepinyldimethylpurine* (48%), m.p. 62–64° (Found: C, 63·5; H, 7·8; N, 28·9. $C_{13}H_{19}N_5$ requires C, 63·6; H, 7·8; N, 28·6%).

6,9-Dimethyl-2-(octahydroazocin-1′-yl)purine (*1q*)

Likewise octahydroazocine gave *4-methyl-6-methylamino-5-nitro-2-(octahydroazocin-1′-yl)pyrimidine* (2k) (58%), m.p. 107–109° (Found: C, 55·4; H, 7·8; N, 24·6. $C_{13}H_{21}N_5O_2$ requires C, 55·9; H, 7·6; N, 25·1%) and thence, *5-amino-4-methyl-6-methylamino-2-(octahydroazocin-1′-yl)pyrimidine* (2l) (72%), m.p. 82–84° (Found: N, 26·2. $C_{13}H_{23}N_5$,H_2O requires N, 26·2%). This aminopyrimidine (1 g) and 98% formic acid (15 ml) were boiled under reflux for 4 h. Evaporation gave the crude formyl derivative which cyclized on heating for 40 min in formamide containing a drop of concentrated hydrochloric acid. Refrigeration gave the *octahydroazocinylpurine* (34%), m.p. 79–81° (from light petroleum) (Found: C, 63·8; H, 7·9; N, 26·8. $C_{14}H_{21}N_5$,0·25H_2O requires C, 63·7; H, 8·2; N, 26·6%).

6,9-Dimethyl-2-(morpholin-4′-yl)purine (*1r*)

Similarly, morpholine gave *4-methyl-6-methylamino-2-(morpholin-4′-yl)-5-nitropyrimidine* (2m) (69%), m.p. 194–195° (Found: C, 47·6; H, 5·8; N, 27·7. $C_{10}H_{15}N_5O_3$ requires C, 47·4; H, 6·0; N, 27·7%); *5-amino-4-methyl-6-methylamino-2-(morpholin-4′-yl)pyrimidine* (2n) (91%), m.p. 115–117° (Found: C, 53·7; H, 7·6; N, 31·6. $C_{10}H_{17}N_5O$ requires C, 53·8; H, 7·7; N, 31·4%); and the *dimethylmorpholinylpurine* (52%), m.p. 151–153° (Found: C, 56·3; H, 6·3; N, 29·9. $C_{11}H_{15}N_5O$ requires C, 56·6; H, 6·5; N, 30·0%). pK_a 3·95±0·05 (anal. λ 240 nm).

6,8,9-Trimethyl-2-(piperidin-1′-yl)purine (*1s*)

(A) 2-Chloro-6,8,9-trimethylpurine (1d) (197 mg), piperidine (0·2 ml) and ethanol (10 ml) were heated for 30 h in a sealed tube at 100–110°. The residue from evaporation was extracted with hot benzene and the clarified extract was evaporated to give the *trimethylpiperidinylpurine* (21%), m.p. 112° (from light petroleum) (Found: C, 63·7; H, 7·7; N, 28·5. $C_{13}H_{19}N_5$ requires C, 63·6; H, 7·8; N, 28·5%). M^+ 245.

(B) 6-Amino-4-methyl-6-methylamino-2-(piperidin-1′-yl)pyrimidine (2f) (220 mg), acetic anhydride (125 mg) and benzene (10 ml) were stirred at 20° for 30 min. The residue from evaporation was dissolved in water and decolorized. Evaporation of the aqueous solution gave N-[*4′-methyl-6′-methylamino-2′-(piperidin-1″-yl)pyrimidin-5′-yl*]*acetamide* (2o) (89%), m.p. 169–170° (from cyclohexane) (Found: C, 59·4; H, 7·3; N, 26·6. $C_{13}H_{21}N_5O$ requires C, 59·3; H, 8·0; N, 26·6%). M^+ 263; ν_{max} 1680 (C=O). The above pyrimidine (26 mg), aqueous 5% sodium hydroxide (1 ml), and ethanol (2 ml) were heated under reflux for 5 h. The solution was adjusted to pH 3 with 2M hydrochloric acid and then evaporated to give the trimethylpiperidinylpurine *hydrochloride* (53%), m.p. 259–260° (sealed tube) (from ethanol–ether) (Found: C, 55·5; H, 7·2; N, 24·6. $C_{13}H_{19}N_5$,HCl requires C, 55·4; H, 7·2; N, 24·9%).

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Purine Studies. XVIII*
The *C*- and *N*-Alkylation of Theophylline and Derivatives

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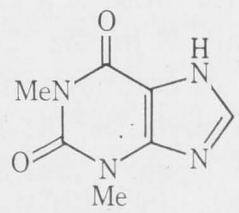
Abstract

Alkylation of theophylline in aqueous alkaline solution with benzyl halides gives, in addition to the expected N 7 benzylated derivative, both 8-benzyl- and 7,8-dibenzyl-theophylline. Synthetic routes to these derivatives have been explored and unusual reactivity associated with benzyl groups in some of the intermediates has been demonstrated. Results which do not preclude the possibility that benzyl radicals could be responsible for the 8-benzylation reaction have been obtained.

Introduction

One currently held view on the mechanism of carcinogenesis is that carcinogens *in vivo* are alkylating (or arylating) agents which interact with the purine bases, adenine and guanine, in nucleic acid. Evidence which supports this theory is that from both *in vivo* and *in vitro* experiments with aromatic carcinogens have been isolated adducts of guanine in which the carcinogen moiety is covalently linked to C 8 of the purine. To explain this, both radical and ionic forms of the carcinogen have been proposed as being the species actually interacting with the purine. Recently a number of papers[1] have dealt with the former aspect; in these the isolation of 8-alkylpurines is described from reactions in which alkyl radicals were generated by various means. On the other hand, two reports have appeared in which 8-alkylation has occurred under conditions which favoured ionic rather than radical type mechanisms. Both cases resulted from attempted N 7 alkylation of the anionic form of theophylline (1).



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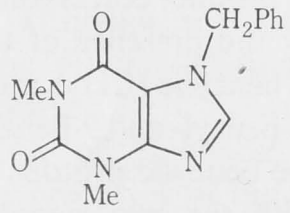

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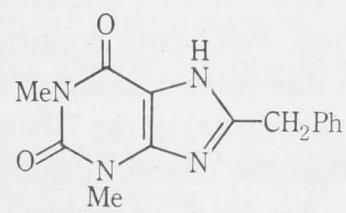

(3)

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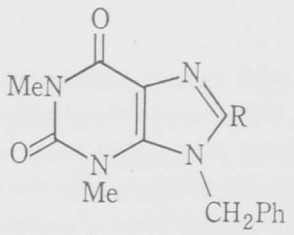
Thus, when crotyl bromide was used only 8-crotyltheophylline[2] was obtained whereas with benzyl chloride a product, claimed to be 8-benzyltheophylline, was isolated[3] in addition to the expected 7-benzyl derivative (2).

Results and Discussion

In view of the discrepancies between the physical data given for the 8-benzyl derivative, noted above, and authentically prepared[4] 8-benzyltheophylline (3) we repeated the benzylation of theophylline, following the published[3] conditions. From the reaction mixture 7-benzyltheophylline (2) crystallized out but the residue, obtained from evaporation of the mother liquors, was separated by preparative t.l.c. into three components, two of which were theophylline and more of the 7-benzyl derivative (2).



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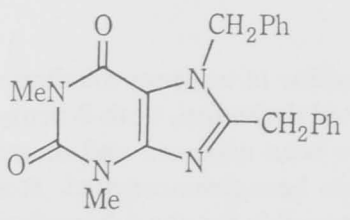

(5)

Table 1. ^{1}H n.m.r. spectra of benzylated xanthine derivatives

δ values in dimethyl sulphoxide

Xanthine	CH_2 (benzyl)	C_6H_5	Me(N 1), Me(N 3)	H 8
1,3-Me_2	—	—	3·47, 3·25	8·05
7-CH_2Ph-1,3-Me_2	5·45 (N 7)	7·30	3·39, 3·19	8·27
8-CH_2Ph-1,3-Me_2	4·05 (C 8)	7·31	3·41, 3·23	—
9-CH_2Ph-1,3-Me_2	5·65 (N 9)	7·30 (m)	3·40, 3·20	7·83
7,8-(CH_2Ph)$_2$-1,3-Me_2	5·55 (N 7) 4·10 (C 8)	7·17	3·40, 3·20	—
7,8-(CH_2Ph)$_2$	5·37 (N 7) 3·92 (C 8)	7·18	— —	—
9-CH_2Ph-8-SCH_2Ph-1,3-Me_2	5·50 (N 9) 4·40 (S)	7·30, 7·0	3·38, 3·23	—
9-CH_2Ph-8-SH	5·83 (N 9)	7·4 (m)	3·40, 3·20	—
1,3-Me_2-8-Ph	—	8·12, 7·48	3·50, 3·27	—

The analytical data for the remaining product corresponded with those of a dibenzylated purine, this being confirmed by the presence of two singlets in the ^{1}H n.m.r. spectrum due to two separate sets of benzylic (CH_2) protons. A comparison carried out with the spectra of the 7-benzyl-, 8-benzyl- and 9-benzyl-theophyllines[5] (4; R = H) showed that the chemical shifts of the benzylic protons of the 7-benzyl and 8-benzyl isomers were in close agreement with the two signals of the dibenzyl derivative (Table 1).

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[4] Hager, G. P., Krantz, J. C., Harmon, J. B., and Burgison, R. M., *J. Am. Pharm. Assoc., Sci. Ed.*, 1954, **43**, 152.
[5] Blicke, E. F., and Schaaf, R. L., *J. Am. Chem. Soc.*, 1956, **78**, 5857.

These findings, together with the absence of any H 8 signal, suggested that the third component was 7,8-dibenzyltheophylline (5); the 8,9-dibenzyl isomer (4; R = CH_2Ph) was ruled out largely on n.m.r. data which showed that in all 9-benzyl-1,3-dimethylxanthines examined extensive multiplicity and splitting of the phenyl protons signal was present in contrast to the singlets found with the 7-benzyl analogues. Confirmation of the assignment of (5) followed from a synthesis involving benzylation of 8-benzyltheophylline, the product being identical (m.p., i.r., ^1H n.m.r., u.v., and t.l.c.) in all respects. The forcing conditions needed to effect alkylation (i.e. benzyl bromide on the sodio derivative in dimethylformamide at reflux temperature) would seem to indicate some degree of steric hindrance being occasioned by the adjacent 8-benzyl and 6-carbonyl groups. In further investigations benzylation of the 7-benzyl purine (2), for which anion formation is precluded, was attempted under a variety of conditions. These included benzyl chloride in aqueous sodium hydrogen carbonate (reflux, 15 h) and dimethylformamide (160°, 6 h and 24 h) but in no case could any dibenzylated derivative be isolated, with only starting material being recovered.

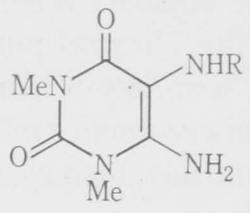
Because it was found that both 7- and 8-benzyl isomers had very similar R_F values in the systems being used, a re-examination of the 7-benzylated product was made. On digestion of this with sodium hydroxide solution and acidification of the filtrate a precipitate of 8-benzyltheophylline was isolated. The claim of the original workers[3] to have obtained 8-benzyltheophylline as the second product of benzylation of theophylline is therefore, in part, correct but in view of the m.p. given, 248–249° (cf. lit.[4] 289–290°), we suggest that contamination by both 7,8-dibenzyltheophylline and unchanged theophylline was present and that a fortuitous ratio of these in the mixture could have given rise to an elementary analysis coincident with that of the monobenzylated derivative.

Attempts at *de novo* syntheses of either 7,8- or 8,9-dibenzyltheophylline have so far been unsuccessful largely due to the unusual benzyl group reactivity shown by some key pyrimidine intermediates. For the former purine (5) the 6-amino-5-benzylaminopyrimidine (6; R = CH_2Ph) intermediate was obtained, with difficulty, by catalytic reduction of the Schiff base derived from the 5,6-diamino derivative (6; R = H) and benzaldehyde. All attempts to cyclize (6; R = CH_2Ph) to the 7,8-dibenzyl purine, using either phenylacetic acid or phenylacetamide, only resulted in N 5 debenzylation and formation of 8-benzyltheophylline (3). Even more unexpected was the isolation of 8-phenyltheophylline (7) when benzyl cyanide was the cyclizing reagent; the mechanism of this reaction still awaits clarification. A more hopeful approach, using uracil analogues in the above scheme, gave 6-amino-5-(*N*-benzyl)-phenylacetamidouracil (8). Although hot sodium hydroxide treatment converted this into 7,8-dibenzylxanthine (9) methylation of this purine to the theophylline analogue (5) has not been achieved.

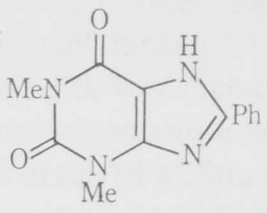
The attempted preparation of 5-amino-6-benzylamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (10a), as a precursor of the 8,9-dibenzyl purine (4; R = CH_2Ph), by various routes gave some unexpected products. Thus, catalytic or chemical reduction of the 6-benzylamino-5-nitropyrimidine (10b) gave 8-phenyltheophylline (7) through cyclodehydration of an intermediate having a reduced form of the nitro group, either the nitroso- or hydroxyamino-pyrimidine, (10c) or (10d). Support for this is the reported[6] isolation of (7) when nitrosation of the 5-unsubstituted 6-benzyl-

[6] Goldner, H., Dietz, G., and Carstens, E., *Justus Liebigs Ann. Chem.*, 1965, **691**, 142.

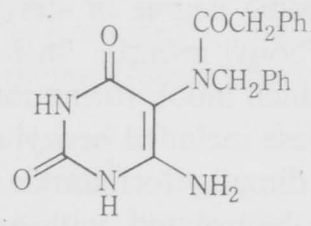
aminopyrimidine (10e) was carried out in the cold. Two further approaches tried, which also gave only the purine (7), were the attempted transamination of the 5,6-diamino (6; R = H) analogue with benzylamine hydrochloride and treatment of the 5-bromopyrimidine (10f) with ethanolic ammonia. In both of these reactions we would suggest a common mechanism operates involving an oxidative cyclization having a 5-amino-6-benzylideneaminopyrimidine as intermediate. A noteworthy point was the debromination of (10f) to give (10e), which occurred with aqueous ammonia; only one previous observation of a dehalogenation of this type in a pyrimidine appears to have been made.[7]



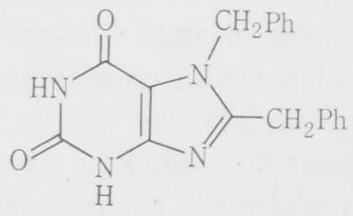
(6)



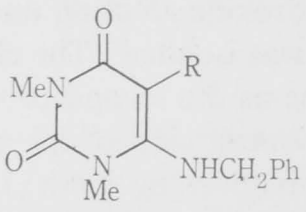
(7)



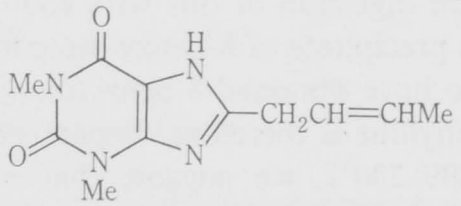
(8)



(9)



(10a) R = NH_2
(10b) R = NO_2
(10c) R = NO
(10d) R = NHOH
(10e) R = H
(10f) R = Br



(11)

With the insight gained from the benzylation work we also repeated the crotyl bromide alkylation. The results of this confirmed the original workers' findings,[2] 8-crotyltheophylline (11) being the sole product obtained; no trace of the 7-crotyl isomer was detected.

Benzylation with Irradiation

In the preceding benzylation of theophylline the purine is reacting in the anionic form and charge localization effects in the imidazole ring must render the 8-carbon atom more nucleophilic and hence more amenable to carbonium ion attack. Alternatively, however, this position is also the most favourable site for radical involvement, irrespective of whether the purine is in an ionized state or as the neutral molecule. In an attempt to gain some insight into the type of reaction obtaining benzylation studies have been initiated, under conditions similar to those given earlier, but in the presence of both short (mainly 254 nm) and long (mainly 360 nm) wavelength u.v. light. Some preliminary results show that under irradiation the yield of 7-isomer decreases to less than half the previous value while the yield of 8-benzyl derivative

[7] Paul, A., and Sen, D., *Indian J. Chem.*, 1963, **1**, 98.

increases tenfold. The significant increase in the yield of 8-benzyltheophylline under these conditions could well indicate that radicals are involved in *C*-benzylation. The fact that only trace amounts of the 7,8-dibenzylpurine were detected may reflect the steric hindrance at N 7 due to the adjacent *C*-benzyl group restricting *N*-alkylation. While the early state of this work does not allow any conclusions being drawn regarding the mechanisms involved in dibenzylation of theophylline, the results thus far do not preclude the idea of an initial 8-benzylation taking place as a result of radical attack, with an ionic alkylation at the 7-position then following under the thermal conditions existing.

Results of the irradiation work will be reported in more detail elsewhere when the studies are completed.

Experimental

Analyses were performed by the Australian National University Analytical Services unit. The n.m.r. spectra were measured in dimethyl sulphoxide at 35° and 60 MHz on a Varian T60A instrument with Me_4Si as an internal standard (chemical shifts in δ) and i.r. spectra were measured in Nujol mulls with a Unicam SP 1000 spectrophotometer. The u.v. source in the irradiation experiments was a 128-W Oliphant chamber reactor equipped with interchangeable short (254 nm) or long (365 nm) wavelength lamps.

Benzylation of Theophylline

To a solution of theophylline (5 g) and sodium hydrogen carbonate (2·3 g) in water (100 ml) was added benzyl bromide (4·7 g) and the mixture heated under reflux for 4 h. The crystalline product, which separated on cooling, was stirred with 1 N sodium hydroxide solution, filtered and recrystallized from ethanol giving 7-benzyltheophylline, m.p. 156–157° (lit.[8] 158°). Acidification of the alkaline filtrate (pH 5) with acetic acid gave a precipitate of 8-benzyltheophylline (0·07 g), m.p. 289–290° (lit.[4] 289–290°). The ethanolic mother liquors from recrystallization of the 7-benzyl derivative were evaporated and the residue taken up in $CHCl_3$ and applied to a preparative t.l.c. plate (Merck type 60 silica) and run in ethyl acetate. Extraction of the lower of the two main bands with hot chloroform gave more of the 7-benzyl isomer (total yield 2·30 g) while from the upper band *7,8-dibenzyltheophylline* (5) (0·04 g), m.p. 120–121°, was recovered (Found: C, 70·3; H, 5·7; N, 15·9. $C_{21}H_{20}N_4O_2$ requires C, 70·0; H, 5·5; N, 15·5%). Evaporation of the aqueous filtrate from the original reaction mixture gave only unchanged theophylline (0·67 g). Virtually identical yields of the 7-, 8- and 7,8-benzylated derivatives, to those given above, were formed when the sodium salt of theophylline and benzyl bromide reacted in water at 100°. In addition to the melting points further confirmation of 7- and 8-benzyltheophyllines was obtained from their spectral comparisons (i.r., ^1H n.m.r.) with those of authentic specimens.

*6-Amino-5-benzylamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione* (*6*; $R = CH_2Ph$)

(A) A solution of 6-amino-5-benzylideneamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione[9] (5 g) in ethanol (150 ml) was hydrogenated over Raney nickel until hydrogen uptake was completed. After removal of the catalyst and evaporation of the solvent the residue was crystallized from ethanol giving *6-amino-5-benzylamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione* (3·5 g), m.p. 184–186° (Found: C, 60·2; H, 5·8; N, 21·8. $C_{13}H_{16}N_4O_2$ requires C, 60·0; H, 6·2; N, 21·5%).

(B) Following Sugimoto and Matsuura's method[10] a mixture of 5,6-diamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (5 g) and benzaldehyde (3·4 g) in ethanol (150 ml) when hydrogenated under the foregoing conditions also gave the same 6-amino-5-benzylaminopyrimidine (m.p., i.r., n.m.r.).

[8] Traube, W., *Justus Liebigs Ann. Chem.*, 1923, **432**, 266.
[9] Traube, W., and Nithack, W., *Ber. Dtsch. Chem. Ges.*, 1906, **39**, 227.
[10] Sugimoto, T., and Matsuura, S., *Res. Bull., Dep. Gen. Ed., Nagoya Univ., Jpn*, 1968, **12**, 24.

7-Benzyltheophylline (*2*)

After heating a solution of the above 5-benzylamino derivative (0·2 g) in formic acid (98%, 15 ml) under reflux for 4 h and evaporation only the *N*-benzyl(pyrimidin-5-yl)formamide was obtained. The crude product was taken up in formamide (5 ml) containing hydrochloric acid (0·1 ml) and heated at 170° (oil bath) for 1 h then evaporated to dryness. On crystallization of the residue from ethanol 7-benzyltheophylline (0·1 g) was obtained, identical (m.p., i.r.) with that derived from benzylation of theophylline, described above.

8-Benzyltheophylline (*3*)

(A) A mixture of 6-amino-5-benzylamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (0·1 g) and phenylacetic acid (0·06 g) was heated at 200° (Wood's metal bath) for 2 h. The product on cooling was 8-benzyltheophylline, m.p. 285° (lit.[4] 289°), from ethanol.

(B) The above product (m.p., i.r.) also resulted when the 6-amino-5-benzylaminopyrimidine and phenylacetamide were fused together (250°) either for short (5 min) or long (3 h) periods.

7,8-Dibenzyltheophylline (*5*)

The sodio derivative of 8-benzyltheophylline, prepared by evaporation of a solution of the purine (1 g) in 1N sodium hydroxide (9 ml) and water (25 ml), was heated (oil bath) with benzyl chloride (0·8 ml) in dimethylformamide (25 ml) under reflux for 4 h. After removal of the solvent the residue was crystallized from ethanol to give 7,8-dibenzyltheophylline (0·7 g), identical (m.p., i.r., n.m.r.) with that previously described.

6-Benzylamino-1,3-dimethyl-5-nitropyrimidine-2,4(*1*H,*3*H)-*dione* (*10b*)

Benzylamine (12·4 g) was added to a well stirred solution of 6-chloro-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione[11] (13 g) in ethanol (150 ml). After 3 h the precipitate was filtered off and suspended in water (100 ml) and the mixture extracted with chloroform (3 × 25 ml) and the extracts dried (Na_2SO_4). The solid remaining on evaporation of the chloroform was crystallized from ethanol giving the *6-benzylamino-5-nitropyrimidine* (11 g), m.p. 140–141° (Found: C, 53·5; H, 4·8; N, 19·2. $C_{13}H_{14}N_4O_4$ requires C, 53·8; H, 4·8; N, 19·3%).

8-Phenyltheophylline (*7*)

(A) The melt, resulting from heating a mixture of the 4,5-diaminopyrimidine (6; R = H) (0·2 g), benzylamine hydrochloride (0·16 g) and benzylamine (10 ml) for 4 h at 150° (metal bath), when cooled and triturated with water gave 8-phenyltheophylline (0·12 g),[2,9] m.p. >350°, in crystalline *form* from acetic acid (Found: C, 60·8; N, 4·8; H, 21·6. $C_{13}H_{12}N_4O_2$ requires C, 60·9; H, 4·7; N, 21·9%).

(B) A solution of 6-benzylamino-1,3-dimethyl-5-nitropyrimidine-2,4(1*H*,3*H*)-dione (5 g) in ethanol (250 ml) was hydrogenated to completion over Raney nickel catalyst. The product (2·4 g) remaining after removal of catalyst and solvent evaporation was 8-phenyltheophylline (i.r., n.m.r.). The same purine was identified as product when either acid stannous chloride or alkaline ferrous sulphate was used as reducing agent for the nitropyrimidine.

(C) Under reflux conditions (4 h) 6-benzylamino-5-bromo-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione[12] (10f) (0·2 g) in saturated ethanolic ammonia solution was converted into 8-phenyltheophylline (0·1 g).

(D) When 6-amino-5-benzylamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (6; R = CH_2Ph) (0·1 g) and benzyl cyanide (10 ml) were heated at 270° for 4 h, then cooled, a yellow solid emerged, identified by i.r. and n.m.r. as 8-phenyltheophylline.

N-(*4-Amino-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-5-yl*)-N-*benzylphenylacetamide* [*6-Amino-5-*(N-*benzyl*)*phenylacetamidouracil*] (*8*)

Under fusion conditions (230°, 4 h) 6-amino-5-benzylaminouracil[8] (0·1 g) and phenylacetic acid (0·72 g) gave the *phenylacetamido* derivative (0·6 g), m.p. >300°, obtained pure by reprecipitation

[11] Bühler, E., and Pfleiderer, W., *Chem. Ber.*, 1966, **99**, 2997.
[12] Bredereck, H., Herlinger, H., and Resemann, W., *Chem. Ber.*, 1960, **93**, 236.

from alkali with acetic acid (Found: C, 65·1; H, 5·0; N, 15·5. $C_{19}H_{18}N_4O_3$ requires C, 65·1; H, 5·1; N, 16·0%).

7,8-Dibenzylxanthine (*9*)

Heating a solution of the foregoing 5-(*N*-benzyl)phenylacetamido derivative (1 g) in 2N sodium hydroxide for 8 h, followed by neutralization with acetic acid (pH 6), gave *7,8-dibenzylxanthine* (0·5 g), m.p. 270° (dec.) (Found: C, 66·9; H, 4·9; N, 16·2. $C_{19}H_{16}N_4O_2,\frac{1}{2}H_2O$ requires C, 66·9; H, 5·0; N, 16·4%).

9-Benzyl-8-benzylthio-1,3-dimethylxanthine (*4*; $R = SCH_2Ph$)

A solution of 9-benzylxanthine-8-thione[5] (4; R = SH) in 5N sodium hydroxide (10 ml) was treated dropwise with stirring with benzyl bromide (0·4 ml). After stirring for 1 h the precipitate was removed and crystallized from aqueous ethanol giving the *8-benzylthio* derivative (0·44 g), m.p. 179–181° (Found: C, 64·2; H, 5·4; N, 14·1. $C_{21}H_{20}N_4O_2S$ requires C, 64·2; H, 5·1; N, 14·2%).

*Reaction of Aqueous Ammonia with 6-Benzylamino-5-bromo-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione*

The 5-bromopyrimidine (0·2 g) in aqueous ammonia solution (25% w/v) was heated under reflux for 4 h. On cooling 6-benzylamino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione[6,7] (0·12 g) (i.r., n.m.r.) was obtained.

Action of Crotyl Bromide on Theophylline

Sodium theophyllinate (1 g) in water (50 ml) was stirred with crotyl bromide (0·66 g) for 6 h. Crystallization of the precipitate from methanol gave 8-crotyltheophylline (0·5 g), m.p. 232–234°; n.m.r. (Me_2SO) 1·60 (m, C–Me), 3·20, 3·38 (s, N–Me), 3·38 (s, CH_2), 5·56 (m, CH=CH). Evaporation of the aqueous filtrate from the reaction mixture gave only unchanged theophylline (0·12 g) and the methanolic mother liquors from the crystallization were found to contain only minor amounts of 8-crotyltheophylline. All the precipitated 8-crotyl purine was alkali soluble, i.e. no 7-alkylated isomer was present. This was confirmed by t.l.c. examination of the products. These data, therefore, verify the findings of original workers[2] for this *C*-alkylation reaction.

Acknowledgments

We thank Dr D. J. Brown for helpful discussions; Dr M. D. Fenn for determination of the n.m.r. spectra; and the Australian National University for supporting K.B. as a Scholar.

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